THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FIFTY-THIRD YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and suc-

cessors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or

any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1940.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1940, the book value of the Endowment Fund in the hands of the Central Hanover Bank and Trust Company, as Trustee, was

General Fund,	Securities (market $807,499.95)	$856,629.50
	Interest in Real Estate	24,921.89
	Cash	30,648.97
		$912,200.36
Library Fund,	Securities (market $150,077.06)	$164,294.79
	Real Estate	20,000.00
	Cash	10,862.62
		$195,157.41

The income collected from these funds during the year was:

General Endowment	$35,674.49
Library	8,463.30
	$44,137.79

The income in arrears on these funds at the end of the year was:

Arrears General Fund	$12,253.69
Arrears Library Funds	4,325.00
	$16,578.69
Arrears at the end of 1939	$15,322.81
a falling behind of	$ 1,255.88

General Biological Supply House, Inc.: The dividends from the General Biological Supply House, Inc. totalled $18,542.00, an increase of $3,556 over 1939.

Bar Neck Property: The rental from the Bar Neck property which is based on the net profit of the garage was $5,097.64, an improvement of about $1,500 over the prior year, during which the absence of the drawbridge adversely affected the business of the garage.

In addition, the notes given for the acquisition of the Bar Neck property are now paid off so that the entire income from this property can now be used for current expenses of the Laboratory.

Retirement Fund: A total of $3,710 was paid in pensions and $923.20 advanced from current funds in prior years was repaid. This fund at the end of the year consisted of:

Participations in mortgages	$ 8,154.39
Interest in Real estate	2,301.88
Cash	5,048.06
	$15,504.33

Plant Assets: The land (exclusive of Gansett and Devil's Lane Tracts), the buildings, equipment and library represent an investment

of	$1,867,005.60	
less reserve for depreciation	564,225.03	
or a net of		$1,302,780.57

Income and Expenses: Income exceeded expenses (including depreciation of $25,648.22) by $8,035.14.

There was expended from current funds for plant account a net of $16,702.27 and in payment of note indebtedness $3,500, and $2,500 was transferred to the Reserve Fund.

The total damage caused by the hurricane on September 21, 1938, finally liquidated during 1940 was $30,152.47
of which 20,000.00
was met by the grant of the Carnegie Corporation (1939) and the balance of $10,152.47 was paid from current funds or charged off.

At the end of the year the Laboratory had no indebtedness on notes or mortgages. It owed on accounts payable $3,689.51, against which it had accounts receivable of $11,667.91 and cash in its general bank accounts of $13,359.26.

The Rockefeller Foundation made a grant of $110,400 for an addition to the library. During 1940, there was received $64,776.62 on this grant, of which $39,851.12 was expended in the year.

Following is the balance sheet, the condensed statement of income and outgo and the surplus account all as set out by the auditors.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1940

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee —Schedules I-a and I-b		$1,107,357.77	
Securities and Cash—Minor Funds—Schedule II		9,194.41	$1,116,552.18
Plant Assets:			
Land—Schedule IV	$ 111,425.38		
Buildings—Schedule IV	1,277,685.06		
Equipment—Schedule IV	179,181.15		
Library—Schedule IV	298,714.01		
	$1,867,005.60		
Less Reserve for Depreciation	564,225.03	1,302,780.57	
Cash in Building Fund		24,925.50	
Cash in Reserve Fund		2,524.65	1,330,230.72
Current Assets:			
Cash		$ 13,359.26	
Accounts-Receivable		11,667.91	
Inventories:			
Supply Department	$ 38,976.75		
Biological Bulletin	11,069.82	50,046.57	

Investments:			
Devil's Lane Property	$ 45,099.78		
Gansett Property	6,030.81		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	17,770.00		
Securities, Real Estate, and Cash —Retirement Fund—Schedule V	15,504.33	97,104.92	
Prepaid Insurance		3,445.81	
Items in Suspense		172.40	$ 175,796.87
Liabilities			
Endowment Funds:			
Endowment Funds—Schedule III	$1,105,900.37		
Reserve for Amortization of Bond Premiums	1,457.40	$1,107,357.77	
Minor Funds—Schedule III		9,194.41	$1,116.552.18
Plant Liabilities and Surplus:			
Donations and Gifts—Schedule III		$1,104,666.73	
Other Investments in Plant from Gifts and Current Funds		225,563.99	$1,330,230.72
Current Liabilities and Surplus:			
Accounts—Payable		$ 3,689.51	
Current Surplus—Exhibit C		172,107.36	$ 175,796.87

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1940

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 35,674.49		$ 35,674.49
Library Fund		8,463.30		8,463.30
Instruction	$ 10,283.63	9,670.00	$ 613.63	
Research	6,086.18	15,068.00		8,981.82
Evening Lectures	32.90		32.90	
Biological Bulletin and Membership Dues	8,834.37	9,725.93		891.56
Supply Department—Schedule VI	27,345.79	32,376.45		5,030.66
Mess—Schedule VII	25,081.97	24,227.77	854.20	
Dormitories—Schedule VIII	23,658.88	13,060.53	10,598.35	
(Interest and Depreciation charged to above 3 Departments—See Schedules VI, VII, and VIII)	24,040.85			24,040.85

Dividends, General Biological Supply House, Inc.		18,542.00		18,542.00
Dividends, Crane Company		400.00		400.00
Rents:				
Bar Neck Property		5,097.64		5,097.64
Howes Property	117.80	160.00		42.20
Janitor House	24.07	360.00		335.93
Danchakoff Cottages	307.77	715.00		407.23
Sale of Library Duplicates		80.26		80.26
Apparatus Rental		1,226.53		1,226.53
Sundry Income		57.50		57.50
Maintenance of Plant:				
Buildings and Grounds	25,121.47		25,121.47	
Chemical and Special Apparatus Expense	15,833.22		15,833.22	
Library Expense	7,675.89		7,675.89	
Workmen's Compensation Insurance	538.14		538.14	
Truck Expense	466.96		466.96	
Bay Shore Property	77.40		77.40	
Great Cedar Swamp	19.20		19.20	
General Expenses:				
Administration Expense	12,426.64		12,426.64	
Endowment Fund Trustee and Safe-keeping	1,014.45		1,014.45	
Interest on Notes—Payable	87.50		87.50	
Bad Debts	228.66		228.66	
Reserve for Depreciation	25,648.22		25,648.22	
	$166,870.26	$174,905.40	$101,236.83	$109,271.97
Excess of Income over Expense carried to Current Surplus—Exhibit C	8,035.14		8,035.14	
	$174,905.40		$109,271.97	

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1940

Balance, January 1, 1940		$163,206.29
Add:		
Excess of Income Over Expense for Year as shown in Exhibit B	$ 8,035.14	
Reserve for Depreciation Charged to Plant Funds	25,648.22	
Transfer from Reserve for Repairs and Repacements on account of Hurricane Water-Damage	1,072.68	$ 34,756.04
		$197,962.33

Deduct:			
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,			
Buildings	$ 1,426.71		
Equipment	5,465.65		
Library	10,104.08	$16,996.44	
Less Received for Plant Assets Disposed of	$ 162.43		
Loss on Equipment Charged Off Due to Hurricane Water-Damage	131.74	294.17	
		$16,702.27	
Payment of Plant Note—Payable		$ 3,500.00	
Transfer to Plant Reserve Fund		2,500.00	
Pensions Paid	$ 3,710.00		
Less Retirement Fund Income	557.30	3,152.70	$ 25,854.97
Balance, December 31, 1940—Exhibit A			$172,107.36

Respectfully submitted,

LAWRASON RIGGS, JR.,
Treasurer.

V. REPORT OF THE LIBRARIAN

The $18,850.00 appropriated for the Library by the Marine Biological Laboratory for 1940 was expended before the end of the year for books, $766.28; serials, $2863.57; binding, $957.55; express, $73.97; supplies, $268.85; salaries, $7200.00; back sets, $2653.35; sundries, $11.48; and a reserve fund of $3977.18 was retained to pay for current serials and back sets, ordered, but not yet received on account of the European war conditions. A balance of $77.77 reverted to the Laboratory plus $80.26 from the sale of duplicates by the Library. An examination of our record over the past decade shows a total of $5970.67 that has so reverted, $4419.49 from the ten yearly budgets assigned by the Executive Committee to Library expenditures and $1551.18 from the Library's sale of duplicates. This is an average reversion of $597.00 for each of the ten years. The explanation to account for a variation from year to year from overspending to underspending is too various to give here, but in general, it is due to caution taken to buy only under advantageous conditions. By calling attention to the sum that has reverted to the Laboratory during this ten-year period, it is the hope of the Librarian that on the occurrence of a very favorable opportunity for buying, the Laboratory will meet this opportunity by placing the necessary sum at the dis-

posal of the Library. It would, in fact, be a fine idea to begin a reserve fund for this purpose.

The Woods Hole Oceanographic Institution appropriation, which is outright and is carried over from one year to the next, was, for 1940, $600.00, plus $82.42 remaining from 1939. Of this $555.11 was expended and reported to the Director. A delayed set ordered from England in August at 50 pounds has arrived in March 1941, before this report goes to press.

In connection with plans made for extending the Library volumes into the addition (the moving will be completed before the end of April, 1941), a count of periodical sets gave the interesting figure of a total of 2366 titles. (These will require the shelving space of the entire top floors, now the fourth and third, and the new section of the reading-room floor, now the second floor. Books will remain in the old part of the second floor and the reprints will extend throughout the first floor.) Of these 2366 titles, only 1257 are received currently; 422 are subscriptions, 403 (15 new) of the Marine Biological Laboratory and 39 (0 new) of the Woods Hole Oceanographic Institution; 609 are exchanges, 544 (6 new) with the "Biological Bulletin" and 65 (1 new) with the Woods Hole Oceanographic Institution publications; 195 are gifts to the former institution and 11 to the latter. An unusual number of books, 142, was bought by the Marine Biological Laboratory Library as selected by the investigators from a list accumulated by the Librarian throughout the previous five years, while the Woods Hole Oceanographic Institution purchased 12; authors presented 8 books to the Marine Biological Laboratory and 2 were received on exchange; publishers gave 43, and the Woods Hole Oceanographic Institution received 2 books from publishers. The record for the filling in of back sets shows 22 completed; purchased by the Marine Biological Laboratory, 15, and by the Woods Hole Oceanographic Institution, 2, by exchange with the "Biological Bulletin," 3, by exchange of duplicates 1, and by gift, 1; partially completed back sets total 49; by purchase, for the Marine Biological Laboratory, 18, for the Woods Hole Oceanographic Institution, 2, by exchange with the "Biological Bulletin," 3, by gift to the former institution, 2, to the latter, 1, and by exchange of duplicates, 23. Reprint additions number 3528; 1614 current of 1939, 637 of 1940, and 1277 of previous dates. The present holdings of the Library number 47,697 bound volumes and 116,305 reprints.

VI. THE REPORT OF THE MANAGING EDITOR OF THE BIOLOGICAL BULLETIN

The *Biological Bulletin* is the property of the members of the Corporation of the Marine Biological Laboratory. It is the one tangible return which they receive in exchange for their membership fees, whether they return each year to the laboratory or not. It seems proper that you should receive, at least from time to time, some report on its progress, its policies and problems.

The annual reports of the Laboratory contain only occasional references to the *Bulletin,* recording appointments to the editorial board and such matters of fact. Fortunately there is preserved in the Tenth Report, for the years 1903–1906, a prospectus issued in 1902 announcing the resumption of publication of the *Bulletin,* which had been interrupted for a year. With the completion of the eightieth volume, after thirty-nine years of continuous publication, it is not unfitting to reexamine this prospectus and see to what extent its promise has been realized.

The *Biological Bulletin* was preceded by the *Zoological Bulletin,* of which two volumes were published in 1897 and 1898 under the editorship of Professor C. O. Whitman, then Director of the Laboratory, and Professor W. M. Wheeler. It was intended to be a companion journal to the *Journal of Morphology* and to take in shorter papers with simple illustrations, where relatively rapid publication of original contributions was desirable. Following the completion of two volumes, it was succeeded in 1899 by Volume I of the *Biological Bulletin.* The title page of the new *Biological Bulletin* shows it to have been edited by the Director and members of the staff of the Marine Biological Laboratory. Volume II was completed in June, 1901.

After the lapse of a year, Volume III appeared, bearing the title: *Biological Bulletin of the Marine Biological Laboratory, Woods Hole, Mass.,* thus clearly establishing the relation of the publication to the Laboratory. The treasurer's report for 1902 shows also that for the first time the relationship was a material one. The editorial staff consisted of E. G. Conklin, The University of Pennsylvania; Jacques Loeb, The University of Chicago; T. H. Morgan, Bryn Mawr; W. M. Wheeler, The University of Texas; C. O. Whitman, The University of Chicago; E. B. Wilson, Columbia University; and Frank R. Lillie appeared for the first time as Managing Editor. Dr. Lillie continued to edit the *Bulletin* for 25 years, when in 1927 he was succeeded by Dr. Carl R. Moore. With two other members of the original staff he still serves as a member of the editorial board.

The prospectus announcing the resumption of publication stated that:

"The Bulletin will be published as heretofore, under the auspices of the Marine Biological Laboratory, and its scope will include Zoology, General Biology, and Physiology. It will contain original articles in these fields and also occasional reviews, and reports of work and lectures at the Marine Biological Laboratory. Preliminary statements of important results will be made a special feature. The Bulletin will be open, as heretofore, to contributions from any source."

I think it is fair to say that the scope of the *Bulletin* still includes Zoölogy, General Biology, and Physiology and that the character of its contents remains essentially unchanged except as it reflects changing emphasis and new developments within these fields. The question of scope is, nevertheless, one of the most perplexing problems of policy with which the managing editor must deal. The objects of the Laboratory and the curiosity of its clientele are broad, and it seems proper that the contents of the *Bulletin* should reflect this catholic interest. It is not undesirable in this day of specialization that one journal at least should present a rather broad cross-section of biology as a whole. On the other hand, practical considerations require some limitation, even within the fields specified in the prospectus, and there is no easier way for an editor to dispose of an unwelcome manuscript than to rule it inappropriate. There is also an evil temptation which besets every editor to allow the selection of papers to reflect too closely his own particular interests and prejudices. I think that in the selection of papers, the guiding principle should be to produce a journal which will interest as widely as possible those who support it with their subscriptions. These are primarily the members of this Corporation, and secondarily, the biological libraries throughout the world. The character of both of these groups argues for as great a diversity as possible. Worthy papers differ greatly in the breadth of their interest. Many papers contain material of value to specialists which are of little interest to other biologists. Where specialists maintain their own journals, as is true, for example, in mammalian physiology, or genetics, it seems preferable that such papers should be published in these places. For many years it was the policy of the *Bulletin* not to publish taxonomic papers since taxonomy was not a part of the program of the Marine Biological Laboratory. However, I feel that today those who are still following the older disciplines deserve some encouragement and that the policy of the *Bulletin* should be more lenient, at least in connection with papers dealing with the local fauna and with groups of organisms which are not covered by specialized journals.

It is significant that the founders of the *Biological Bulletin* did not call it the *Marine Biological Bulletin*, though it was published under the auspices of a marine laboratory. It is evident that they came to Woods Hole not so much to study the sea, but because they found there good material for the study of more general problems, and that they intended that the *Bulletin* should represent all aspects of biology. In this characteristic there has been no change. It is, however, natural that the journal should deal to a large extent with the biology of marine organisms since these are the creatures on which many of your studies are made, and it seems reasonable to assume that you will be interested in papers of all sorts dealing with these organisms and with the waters in which they live. Thus the *Bulletin* should retain a distinctly salty flavor and should welcome especially work done at other marine stations.

The prospectus states that the *Bulletin* will be open to contributions " from any source." An examination of 778 papers published between 1930–1940 shows that 29 per cent originated from this Laboratory; 22 per cent were contributions from other marine laboratories, including 9 per cent derived from the Woods Hole Oceanographic Institution These included contributions from: the Mt. Desert Island Biological Laboratory, the Scripps Institution of Oceanography, the Duke University Marine Laboratory, the William Kerckhoff Marine Biological Laboratories of the California Institute of Technology, the Hopkins Marine Station, the U. S. Bureau of Fisheries, the Atlantic Biological Station at St. Andrews, the Chesapeake Biological Laboratory, the University of Washington Oceanographic Laboratories, and the New York Aquarium; the Naples Zoölogical Station, the Marine Biological Laboratory at Plymouth, the Bermuda Biological Station, the Bergens Museum Biologiske Stasjon, the Pacific Biological Station at Nanimo, and the Misaki Marine Biological Station at Kanagawa-ken. The remaining 49 per cent came from various university laboratories and may in part have been based on work done here or at other marine stations. This distribution conforms well to the principles discussed above.

While the bulk of the material in the *Bulletin* consists of original articles, some attempt was made a few years ago to print the lectures given at the Laboratory. Because of the difficulty in securing the manuscripts and also because of the general pressure for space, this practice was discontinued. The publication of reviews has not been made a feature of the *Bulletin* and there is probably no reason why it should in view of the special journals now devoted to this purpose. An occasional paper bringing together material of particular local interest would be of value to workers in the Laboratory. I have in mind as an example Dr. Harvey's paper on " Physical and chemical constants of the egg of

Arbacia punctulata" (*Biological Bulletin*, April, 1932). The promise that preliminary statements of important results would be made a special feature of the *Bulletin* is now fulfilled by the publication of abstracts of the papers delivered at the scientific meetings during the summer.

The prospectus continues:

"There is in America no journal that takes the place of the *Biologisches Centralblatt* or the *Anatomischer Anzeiger* in Germany, although there is abundance of material to support such a publication. It is hoped that the Bulletin may occupy this field, and meet the need for rapid publication of results; the editors, therefore, undertake to issue one number each month, making two volumes a year, if the material offered is sufficient."

At the time the *Bulletin* was founded, the American journals participating in its field were: the *Journal of Morphology*, the *American Journal of Physiology*, the *Annals of Botany*, the *American Naturalist*, the *Botanical Gazette* and the *Journal of Comparative Neurology*. The establishment shortly thereafter of the *Anatomical Record*, the *American Journal of Anatomy* and the *Journal of Experimental Zoology*, and the subsequent appearance of many others has largely eliminated this special raison d'être. The hope that the *Bulletin* might meet the need for rapid publication of results has not always been fulfilled, for it was recorded in the twenty-seventh report, for the year 1924, that ". . . lately, in common with many other biological research journals, it had been falling behind in promptness of publication so that about a year elapsed between the receipt of manuscripts and their appearance in print." In the hope of correcting this situation the *Bulletin* was enlarged to 900 pages and the subscription price increased from $6.00 to $9.00 per year, and the fees of members of the Corporation were increased proportionately. This change reduced the time required for publication to at most six months. At the present time the *Bulletin* is run on the theory that the only way to ensure prompt publication is to accept for publication no more papers than one can afford to publish at once. A journal operating on a fixed income has no more chance of catching up with an accumulation of papers it cannot afford to publish at once than does a man in like circumstances who has allowed himself to get some months behind in paying his bills. It is better to require a few papers less suited to one's purpose to find a publisher elsewhere than to delay the entire stream of publication chronically. During the past 11 years no paper has remained in the editor's hands more than two months before going to the printer except under special circumstances, as when occasionally delays occur in securing the necessary reports from

referees. In order to facilitate this practice, it is customary not to arrive at a decision concerning the disposition of any paper until the accumulated material can be considered together at the time of going to press.

The *Bulletin* was originally offered at a price of $3.00 per volume of 300 pages. It is interesting to note that this is exactly the cost to the subscriber per page of the present issues. Because of the low price, the length of articles was originally limited to 25 pages and lithographic prints were excluded. The cost of illustrations above $10 was charged to the author. These limitations are no longer exactly exercised though, naturally, longer papers must be discouraged if opportunities for publication are to be widely distributed. The criterion is that no paper should be longer than is necessary to adequately present its contribution and short and long papers alike should be scrutinized from this point of view. This is sometimes an unwelcome task, but on the whole, I have found our contributors uncommonly coöperative and goodnatured. They can usually be made to appreciate that concise presentation is read with understanding. It is no longer the practice to charge authors for a part of the cost of necessary illustration provided they are content with line cuts and halftones and comply with the general principles discussed above.

The prospectus closes:

"The *Bulletin* will undoubtedly meet a real need; but the responsibility for its success rests with American biologists, and the editors, therefore, confidently appeal to them for their support. This can most practically be given in the two forms of subscriptions and contributions to its pages." The need for the *Bulletin* and its success, as well as the support which it has received from American biologists, is amply attested by its contents. The Index which was published at the completion of Volume LX listed approximately 1200 titles. An index which is now being prepared of the last 20 volumes records an additional 660 titles of original articles and 652 titles of abstracts, making a total of some 2500 contributions. An examination of the original articles appearing between 1930–1940 showed that 40 per cent of these were written by members of the Corporation. The list of Corporation members contains very few productive workers in fields appropriate to the *Bulletin* who are not contributing to its pages.

On the financial side, the *Bulletin* is supported in three ways. Of 1100 subscriptions, 300 go to members of the Corporation in return for their membership fees. The remaining subscriptions are divided about equally between paid subscriptions from libraries and exchanges. The treasurer's reports show that with this support the *Bulletin* just about

breaks even or sometimes shows a small profit. This is due in a sense to the accountant's art and requires a word of explanation. The income from exchanges represents a transfer of Library funds to the *Bulletin* in payment for issues used to secure exchanges. Thus, in a sense, the Library helps to subsidize the *Bulletin* and the sum involved does not represent cash income. In return, however, the Library receives 656 items in exchange, or approximately one-half of its current list of periodicals. It is this fortunate association of the *Bulletin* with the Library which enables it to make ends meet. The large number of exchanges greatly widens the distribution of the papers published in the *Bulletin*.

Respectfully submitted,

ALFRED C. REDFIELD,
Managing Editor.

VII. THE REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen: I present herewith a report of the fifty-third session of the Marine Biological Laboratory, for the year 1940.

1. *Present Conditions.* The grievous conditions which are now disturbing the world have thus far affected but little the activities of this Laboratory. In the season just past investigators occupied almost all of the available space, and students filled the courses. The attendance was practically equal to that of 1937, the largest in the history of the institution. Except for an occasional lack of current foreign journals, and of scientific apparatus usually purchased abroad, we continued our work in 1940 without serious difficulty. This fortunate state of affairs cannot be expected to continue. Evidences of the change are already apparent. During the season of 1941 several of our investigators will give up their usual lines of research and will work at home on problems connected with national defense, while others will carry on similar research at Woods Hole. Apparatus, formerly obtained without delay, is now difficult if not impossible to secure. Thus far, the Government has made no request for space and facilities to be devoted to defense measures. We shall continue our usual activities of instruction and research so long as no emergency arises which might temporarily interrupt them.

2. *The Library Addition.* We may congratulate ourselves that in spite of the uncertainties which have surrounded us during the past year, we have completed one part of the extensive building program which was

discussed at the last Trustees' meeting. The generosity of the Rockefeller Foundation made possible the immediate erection of the much-needed extension of our Library. It will be recalled that, under the terms of the gift of $110,400, the Laboratory accepted the responsibility of obtaining from other sources at least $25,000 to be used primarily for filling gaps in the present files of journals. A most liberal interpretation of these terms permitted us to proceed with actual construction before this sum was in hand.

Work was begun in August, 1940 under the direction of the architect, Mr. F. V. Bulfinch. On his advice, the Executive Committee voted to give the contract for the building to the Sawyer Construction Company of Boston, without waiting for bids to be submitted by other companies. The wisdom of this action was at once apparent, for excavation began immediately, even before the architects' plans were fully completed. The structural steel and other essential materials were obtained quickly and without difficulty. Had we waited six weeks or more for competitive bidding to be completed, it is very probable that their delivery would have been greatly delayed, if not indefinitely postponed. The construction company awarded contracts, after competitive bidding, for much of the inside work, such as the plumbing, heating, electrical installations, and the book stacks. The building was enclosed before cold weather set in, and was heated satisfactorily by means of a new oil furnace, a part of the Library project. It may be added that those parts of the Brick Building where heat was required were maintained at a comfortable temperature. The final cost of the building and its equipment was substantially less than the first estimates of the architect and the contractor. With the balance we have been able to rebuild the Eel Pond wall, considerably damaged by the building operations, install an electric book lift in the shaft provided in the old Library but never used, pave the road and parking spaces close to the new wing, and re-grade the lawn.

Those who use the Library will find many improvements over the old conditions. The journals and books are now spread out over three floors only, an arrangement which greatly reduces the amount of stair climbing. In the new wing there are many well-lighted desks, some of which will be reserved for those who spend all of their time among the books, and others which will be for general use. In the basement will be housed, temporarily it is hoped, apparatus for sterilizing glassware, for distilling water and for other services which heretofore have been widely scattered throughout the Brick Building. There are also two dark-rooms.

The space now available will serve the rapidly growing Library for many years. For this happy situation we are deeply indebted to the Rockefeller Foundation which now, as in the past, has so greatly furthered the work of the Laboratory. To the Library Committee and Mrs. Montgomery must be given credit for the planning of the book stacks, the amply spaced study tables, and other features which all readers will appreciate. Finally, thanks are due to Mr. Bulfinch and Mr. Sawyer, who have so successfully designed and constructed the building of which we may well be proud.

We take even greater satisfaction in its contents. The Library is an indispensable part of our equipment. Although the number of volumes is not large, the selection of books and serials has been made with such skill that we now have on our shelves practically all of the important journals, and we are adding to the number every year. In the Annual Report of 1889, a committee consisting of E. S. Minot, W. T. Sedgwick, and C. O. Whitman, made a double appeal ". . . first, to the public, for $1,500 to complete the more important sets of journals, and secondly, to all biologists, for reprints of their articles. We do this with confidence, because we believe that nowhere in this country would such a library be more useful or valuable than in the Marine Biological Laboratory." At that time the library consisted of 343 volumes, 23 reprints, and 23 serials, of which many were not current subscriptions. From this modest beginning it has grown in importance until it is now one of the most important biological libraries in the world. The increase in the number of volumes, serials, and reprints during the past twenty years is shown in the following figures, taken from the Librarian's reports.

	Volumes	Serials	Separates
1920	10,243	153	8,532
1925	15,000	500	25,000
1930	31,510	1,060	66,231
1935	40,180	1,271	91,641
1940	47,897	1,257	116,305

3. *Committee on Instruction.* The primary purpose of this Laboratory is to encourage research by qualified investigators and to increase their number by preparing students to undertake original work. To determine whether the courses of instruction are fulfilling this purpose, the Committee on Instruction, under the able leadership of Dr. Allee, has made a careful study of the situation. They were guided in their deliberations by the following principles adopted by the Executive Committee.

1. That instructorships are to be regarded as aids to research.
2. That the duty of the instructors consists of research and teaching, and that they consequently are to remain in residence for a period longer than the duration of the course.
3. That instructorships should be distributed widely among American institutions of learning.
4. That long tenure of instructorships should be discouraged.
5. That it is better to have instructors who are specialists in the courses in which they teach.
6. That the Director be authorized to appoint a Standing Committee on Instruction to report to the Executive Committee each year.

The Committee met weekly during the summer, discussing these problems with the directors of the various courses and with many other interested investigators. Their general conclusion was that the work of instruction is on a fairly satisfactory basis. The instructors are actively engaged in productive research, many of them in the general subjects which they are called upon to teach. Not all, however, are in residence at Woods Hole for a substantial part of the summer. They are drawn from 23 colleges and universities, of which 12 are eastern, 5 are in the midwest, 4 in the south, and one each from the far west and Canada. The tenure of instructorships averages about 5 years; that of the directors, excluding their previous service as instructors, about 9 years. The Committee felt that a more frequent change of directors would be desirable.

The Committee also recommended that in place of Dr. Irving, who resigned after five successful years as head of the Physiology Course, Dr. A. K. Parpart be appointed. And furthermore, that Dr. Calkins' desire to be relieved of the course in Protozoölogy be granted, and that this course be discontinued. It was with regret that this action was taken. Under Dr. Calkins the Protozoölogy Course has for many years occupied an outstanding place in our summer work of instruction. A large proportion of his students, drawn from all parts of the world, have made significant contributions to biology, a lasting tribute to the training and inspiration which they received from him. The Committee were convinced that no one could replace him, and that since there are now many excellent courses in Protozoölogy offered in various parts of the country, the need for continuing such a course here is not imperative.

I wish to express the thanks of the Trustees and Corporation to the members of this Committee, and to the other Standing Committees who have during the year coped successfully with many difficult problems.

4. *Election of Officers and Trustees.* At the meeting of the Corporation held August 13, 1940 the following Trustees were elected Trustees Emeritus:

Caswell Grave, Washington University
Ross G. Harrison, Yale University
C. E. McClung, University of Pennsylvania

The new Trustees elected at that meeting were:

Dugald E. S. Brown, Class of 1942
C. W. Metz, Class of 1944
H. H. Plough, Class of 1944

5. There are appended as parts of this report:

1. Memorials of deceased Trustees.
2. The Staff, 1940.
3. Investigators and Students, 1940.
4. A Tabular View of Attendance, 1936–1940.
5. Subscribing and Coöperating Institutions, 1940.
6. Evening Lectures, 1940.
7. Shorter Scientific Papers, 1940.
8. The General Scientific Meeting, 1940.
9. Members of the Corporation, 1940.

Respectfully submitted,

CHARLES PACKARD,
Director.

1. MEMORIALS OF DECEASED TRUSTEES

MEMORIAL TO DR. M. M. METCALF

BY DR. R. A. BUDINGTON

It is altogether fitting that the Corporation of the Marine Biological Laboratory, at its annual meetings, should pause to pay such salutation and honor as it may to those recently removed by death, and who over many years supported the Laboratory by scientific work, wise counsel, and energetic endorsement.

Such a Corporation member was Maynard Mayo Metcalf, who died last April 19th after a very prolonged illness, which began suddenly while he was at work in this building. His age was seventy-two years.

Dr. Metcalf's chief biological mentors were Prof. Albert A. Wright at Oberlin (Wright was one of the very early workers at Woods Hole), and Prof. W. K. Brooks of the Hopkins, under whom he took the

doctorate in 1893. His academic appointments as teacher were as organizer and head of the Department of Zoology at Goucher College, 1893–1906; at Oberlin he reorganized the corresponding department and directed it from 1906 till 1914; from 1926 till 1933 he was research associate with rank of Professor at the Johns Hopkins University. During the year 1924–25 he was chairman of the Division of Biology and Agriculture of the National Research Council, Washington.

Among Metcalf's earliest published studies were some on morphological and embryological features of Amphineura and gastropods; but thereafter for several years his attention was given to the morphology, physiology, phylogeny, and taxonomy of the Tunicata with major emphasis on pelagic forms. He presented very comprehensive collections of these to the National Museum. His third and most arduous series of studies dealt with the morphology, taxonomy, and cytology of the Opalinidae; these led him to far-reaching analysis of specific host-parasite relations, with deductions therefrom as to the ancient distribution of Amphibia, as well as to evidences of former land connections between now-separated continents.

All his life an outstanding charaacteristic of Metcalf, which should be mentioned in any summary of his scientific work, was that of giving credit to collaborators. Especially in his later years was assistance necessary; and all such received appropriate acknowledgment in the publications involved.

Metcalf's publications include: papers exceeding 120 in number; a book, "Organic Evolution" (Macmillan); and three large monographic volumes on the opalinids. The most recent of these was issued by the Smithsonian Institution as a Bulletin of the National Museum last spring.

He was elected to membership in 28 American, 3 British, and 3 French learned societies, and was a member of the Authors Club, London. For 45 years he was a summer frequenter of the Woods Hole Laboratories, and a member of the Board of Trustees of the Marine Biological Laboratory from 1896 till his death—44 years. Few men indeed have been as deeply sincere in their solicitude for and belief in the functions of this Laboratory as was Maynard Metcalf. Directly or indirectly he assisted many a student, in financial or other ways, to come here for study and research; and mention should here be made of his gift of his large collection of reprints to our library.

As a man he was chronically of discriminating judgment, positive opinions, and uncompromising integrity. He was thoroughly human of the finest grade; an optimist; an idealist; a dispenser of cheer, with rare generosity of spirit, and capacity for friendship. He will not be forgotten.

Memorial to Dr. H. McE. Knower

By Dr. Ross G. Harrison

Henry McElderry Knower died in Baltimore on January 10, 1940, at the age of 71, after a long and distressing illness, which was borne with the courage, patience, and good humor that characterized his whole life.

He was born in Baltimore on August 5, 1868 and was educated in schools in that city and at the Johns Hopkins University, where he received the A.B. degree in 1890 and the Ph.D. in 1896. After graduation Knower held an instructorship for one year at Williams College and subsequently was for ten years on Doctor Mall's staff in the Department of Anatomy at the Johns Hopkins Medical School. From there he went to the University of Toronto as lecturer in 1909 and the following year to the University of Cincinnati as Professor and Head of the Department of Anatomy. After his resignation in 1924 he served as a visiting professor at the University of Georgia, later as Professor of Anatomy at the University of Alabama (1927–29) and as Associate Professor of Anatomy in the Albany Medical College. His last appointment was that of Research Associate in Biology in Yale University.

Knower spent the summer of 1896 at the Marine Biological Laboratory as an investigator and was elected to membership in the Corporation. The following year he was on the staff of the Invertebrate course. In 1908 he became one of the permanent members of the Woods Hole summer colony and a regular attendant at the Laboratory, until the failure of his health made that impossible. From 1909 till 1919 he served as librarian of the Marine Biological Laboratory, and it was during his administration that the library began its period of rapid growth. It was well arranged and catalogued, particularly after its removal to the Crane Building made that possible.

Knower first became interested in the embryology of termites when in Jamaica as a student. This was the subject of his doctoral dissertation, but on entering the Department of Anatomy at Johns Hopkins in 1899 his interest shifted to the development of the vascular system, the study of which became his life work. Much of his research in this field was done at Woods Hole. He developed very delicate methods of injection and accumulated a great collection of exquisitely injected embryos. Fortunately he was able to complete one of the major installments of this work shortly before his death.

His services to scientific publication in this country were unusual. Through his energy and enterprise the foundation of the *American Journal of Anatomy* was greatly hastened, and its establishment came

just at the time when most needed. Over twenty years of his life were devoted to this undertaking, which he served with devotion and skill as Secretary of the Editorial Board. In 1906 he initiated the publication of the *Anatomical Record*, first as a supplement to the *American Journal of Anatomy* but soon to become an independent journal with its own editorial board.

In all of his relations Knower was steadfast and sincere. He was good humored, sympathetic with youth and wise in his counsel. Through his death many of us here have lost a warm, devoted and genial friend. The Corporation of the Marine Biological Laboratory desire to record their sorrow at his death, their sense of personal loss, and their appreciation of his many contributions to biological science and his services to the institution.

Memorial to Dr. Charles Zeleny

By Dr. Fernandez Payne

Charles Zeleny, Professor of Zoölogy at the University of Illinois, died at his home in Urbana December 21, 1939. He was born at Hutchinson, Minnesota, September 17, 1878, and spent his early boyhood days there. Later his parents moved to Minneapolis where he entered the University of Minnesota and graduated in 1898. He remained as a graduate and received his M.S. in 1901. The next year he was a graduate student at Columbia University, working with T. H. Morgan and E. B. Wilson, and the following year he worked at the Naples Zoölogical Station. Returning to America in 1903, he entered Chicago University where he obtained the Ph.D. in 1904. He came to Indiana University as an instructor in the summer of 1904. Here he advanced rapidly and held the rank of Associate Professor at the time of call to the University of Illinois in 1909. Beginning at Illinois as an Assistant Professor, he was promoted the next year to the rank of Associate Professor and in 1915 to a Professorship. Upon the retirement of Professor H. B. Ward in 1933, he was made head of the Department of Zoölogy and chairman of the Division of Biological Sciences. Because of ill health, he had retired from his executive duties in 1938.

On May 29, 1911, he married Ida Benedicta Ellingson, of St. Morris, Wisconsin. Mrs. Zeleny and a son, Charles, Jr., survive.

Dr. Zeleny's family is unique in that three of his brothers are scientists of note. Anthony Zeleny, now retired, was Professor of Physics at the University of Minnesota; John Zeleny is Professor of Physics at Yale; and Frank Zeleny is an engineer with the Burlington Railway.

As is true with every great man, chronological facts such as those enumerated tell but little of the life of Charles Zeleny. They are cold, external. It was the writer's good fortune to have been a student in Dr. Zeleny's first class in embryology taught at the Biological Station in the summer of 1904. For the next three years, our associations were intimate. We worked together, ate at the same table, played together and tramped through the woods and fields together. The fact that one was teacher, the other student entered but little into our thinking. The friendship formed in those early years remained to the end. As a friend he was true, somewhat reserved, seldom talked of his own personal affairs, possessed a subtle, sometimes mischievous wit, appreciated by those who know him best. Seldom did he complain about anything. Bitterness, if present, was kept hidden.

As a teacher he was kind, helpful, encouraging, stimulating. As a zoölogist his papers in the fields of regeneration, experimental embryology and genetics speak for themselves. They rank among the best contributions of his time. Originality in thinking stands out prominently in all his work.

In recognition of his attainments, he was elected vice-president of section F of the A. A. A. S. in 1932, and president of the American Society of Zoölogists in 1933.

Dr. Zeleny's death at the early age of 61 years is not only a loss to his relatives and friends, but to science.

Memorial to Captain John J. Veeder

By Dr. F. R. Lillie

John J. Veeder, Captain of the fleet of the Marine Biological Laboratory from 1890 to 1933, was born on the island of Cuttyhunk January 27, 1859. Like all Cuttyhunkers, he was accustomed to the management of boats from early years, and acquired a most intimate knowledge of the shoals, tides, currents and weather conditions of Vineyard Sound and Buzzards Bay. He married and moved to Woods Hole in 1881.

The Marine Biological Laboratory was founded in 1888, and as Dr. Bumpus has written me, "The summer of 1890 found the steam launch "Sagitta" proudly added to the fleet of two old green dories that had been inherited from the Annisquam Laboratory." It became necessary to appoint a captain and John J. Veeder was called in for examination by Dr. Gardiner. He was asked to "box the compass." Dr. Bumpus relates, "The speed with which he went through the ritual settled the matter then and there. Captain Veeder was promptly commissioned."

For a year, until George M. Gray was appointed, Captain Veeder acted also as collector; and afterwards collaborated closely with the Supply Department, became thoroughly familiar with the collecting grounds, and located and set the fish traps of the Laboratory.

Captain Veeder was in charge of the class trips and picnics, and though many thousands were carried in the years of his service, no one was ever lost. He was a past master of the technique of the clambakes which added so greatly to the enjoyment of the picnics. He kept his eye on the weather and he always veteod a trip if his extraordinary weather sense and wisdom warned him that the trip would be dangerous. I cannot say how many times he came to the rescue of our amateur sailors in distress, when marooned by bad weather or ignorance of tidal currents; and very frequently he and the crew went to the aid of small craft grounded on shoals in the Hole or near the harbor.

He had the good old Cape Cod dignity and self-respect; he was a shrewd judge of men in all walks of life, and met all on an equal basis. He never regarded his position merely as a job; whatever was "for the good of the Laboratory," as he used to say, was always cheerfully and skilfully performed. He acted as interpreter of the Laboratory to the town folk or in town meetings, and was helpful in maintaining the good relations which we have always valued.

2. THE STAFF, 1940

CHARLES PACKARD, Associate Director, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Emeritus, Columbia University.

E. G. CONKLIN, Professor of Zoölogy, Emeritus, Princeton University.

CASWELL GRAVE, Professor of Zoölogy, Emeritus, Washington University.

H. S. JENNINGS, Professor of Zoölogy, University of California.

FRANK R. LILLIE, Professor of Embryology, Emeritus, The University of Chicago.

C. E. MCCLUNG, Professor of Zoölogy, Emeritus, University of Pennsylvania.

S. O. MAST, Professor of Zoölogy, Johns Hopkins University.

T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.

G. H. PARKER, Professor of Zoölogy, Emeritus, Harvard University.

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II. Instruction

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A. J. Waterman, Assistant Professor of Biology, Williams College.

Junior Instructors

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PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

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EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

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W. W. Ballard, Assistant Professor of Biology and Anatomy, Dartmouth College.
Donald P. Costello, Assistant Professor of Zoölogy, University of North Carolina.
Viktor Hamburger, Assistant Professor of Zoölogy, Washington University.
Oscar Schotté, Associate Professor of Biology, Amherst College.

PHYSIOLOGY

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Grinnell, Stuart W., Research Associate, Swarthmore College.
Gwartney, Richard H., DePauw University.
Hayashi, Teru, Graduate Assistant, University of Missouri.
Hemstead, George W., Student, Union College.
Herskowitz, Irwin, Graduate, Brooklyn College.
Höber, Josephine, Philadelphia, Pennsylvania.
Ito, Tetsuji, Research Fellow, New York University, College of Medicine.
Jacobs, Joye, Assistant, University of Maryland, School of Medicine.
Jakus, Marie A., Graduate Assistant, Washington University.
Jones, William D., Graduate Student, University of Pennsylvania.
Kalmanson, George M., Research Fellow, Washington University.
Keefe, Eugene L., Research Assistant, Washington University.
Klein, Ethel, Research Assistant, University of Pennsylvania.
Lewis, Lena A., Research Assistant, Ohio State University.
McVay, Jean, Assistant, Northwestern University.
Marrazzi, Rose, Herter Fellow in Department of Pharmacology, New York University, College of Medicine.
Martin, Phyllis Cook, Assistant Professor of Biology, Pennsylvania College for Women.
Martin, Rosemary D. C., Assistant in Biology, University of Toronto.
Merwin, Ruth M., Research Assistant in Zoölogy, University of Chicago.
Meyerhof, Bettina, Research Assistant, Johns Hopkins University Medical School.
Milford, John J., Graduate Assistant, New York University.
Neubeck, Clifford E., University of Pittsburgh.
O'Brien, F. Donal, Canisius College.
O'Neal, John D., Graduate Student, University of Pittsburgh.
Papandrea, D. A., Student, Albany Medical College.
Ramsdell, Pauline A., Research Assistant, Johns Hopkins University Medical School.
Rimmler, Ludwig, Jr., Research Assistant, Syracuse University, College of Medicine.
Rollason, H. Duncan, Jr., Williams College.

RONKIN, RAPHAEL R., Student, University of California.
SCHAEFFER, OLIVE K., Research Assistant.
SHELDEN, FREDERICK F., Instructor in Physiology, Ohio State University.
SKOW, ROYCE K., Research Assistant, Stanford University.
SPRATT, NELSON T., JR., Research Assistant, University of Rochester.
TRINKAUS, J. PHILIP, Assistant, Wesleyan University.
WALTHER, ROWLAND F., Research Assistant, Ohio State University.
WELLINGTON, DOROTHY, Research Assistant, New York University.
WILLIAMSON, ROBERT R., Student, The University of Chicago.
WOODWARD, ARTHUR, JR., Teaching Fellow, New York University.
WORKMAN, GRACE, Research Assistant, University of Toronto.
WULFF, VERNER J., Northwestern University.
ZIMMERMAN, ALICE C., Graduate Student, Brown University.

Students, 1940

BOTANY

ANDERSON, JOE N., Student, University of Michigan.
BROWN, DONALD H., Student, Dartmouth College.
BROWN, DOROTHY M., Science Instructor, St. Luke's Hospital, New York City.
BUCHANAN, NATALIE V., Student, Wellesley College.
CAMPBELL, VIRGINIA, Wheaton College.
CIU, RUTH E., University of Michigan.
MACCOSBE, HENRIETTA E., Instructor in Botany and Zoölogy, Pennsylvania State College.
MORGAN, DELBERT T., JR., Kent State University.
SANDERS, JOAN, Smith College.
SILVER, SAMUEL, Graduate Student, College of the City of New York.

EMBRYOLOGY

ALPER, CARL, Student Assistant, Brothers College, Drew University.
ATKINSON, WILLIAM B., Graduate Student, University of Virginia.
BELANGER, LEONARD F., Assistant in Histo-embryology, University of Montreal.
CASS, RUTH E., Instructor, Russell Sage College.
DUBOIS, REBECKAH, Student, Vassar College.
FETTER, DOROTHY, Instructor, Brooklyn College.
FINCKE, ROBERT T., Graduate Teaching Assistant, Indiana University.
FOULKS, JAMES G., Graduate Teaching Assistant, University of Rochester.
FRIEDMAN, ROBERT S., Graduate Student, Harvard University.
GOLDMAN, PHILIP W., Graduate Student, Harvard University.
HALSTED, GEORGE O., Princeton University.
HARTMANN, J. FRANCIS, Assistant in Histology and Embryology, Cornell University.
HARTUNG, ERNEST W., JR., Harvard University.
HEATH, JAMES P., Stanford University.
HENDERSON, JOHN M., McGill University.
HOPPER, ARTHUR F., JR., Laboratory Assistant, Yale University.
JOHNSON, VIRGIL O., Technician, University of Oklahoma.
JOLLY, MARGIE, DePauw University.
JONES, SARAH R., Graduate Assistant, Connecticut College.
KARELSEN, JUNE VAN RAALTE, Undergraduate, Oberlin College.
KRANTZ, MARION, Student, Bennington College.
LEE, RICHARD E., Harvard University.
LUDWIG, FRANCIS W., Villanova College.

McFarland, William, Student, Washington and Jefferson College.
Miller, Gerald, Student, Oberlin College.
Nichols, Myron McCall, Laboratory Assistant, DePauw University.
Pond, Sidney M., Wesleyan University.
Robinson, Edwin J., Jr., Teaching Fellow, Washington Square College, New York University.
Samorodin, Albert J., Graduate, University of Minnesota.
Sawyer, Charles H., Assistant in Biology, Yale University.
Sherman, Frederick G., Laboratory Assistant, Northwestern University.
Steele, Kenneth C., Dartmouth College.
Sweeny, Frank P., Amherst College.
Yancey, Maude J., Student, North Carolina College for Negroes.

PHYSIOLOGY

Baylor, Edward R., Student, University of Illinois.
Blanchard, Barbara D., Teacher, Placer Junior College, California.
Carleen, Mildred H., Graduate Assistant, Mount Holyoke College.
Chidsey, Jane L., Assistant Professor, Wheaton College.
Davies, Philip W., Johnson Foundation Scholar, University of Pennsylvania.
Edgerley, Robert H., Graduate Assistant, Ohio State University.
Edwards, George A., Graduate Assistant, Tufts College.
Everett, Guy M., Graduate Teaching Assistant, University of Maryland Medical School.
Fox, Ruth P., Assistant, Vassar College.
Henry, Richard J., University of Pennsylvania, School of Medicine.
Hohwieler, Harold J., Graduate Assistant, Washington University.
Holton, George W., Wesleyan University.
Jackson, Blanche E., Fellowship Student, Radcliffe College.
Jakus, Marie A., Graduate Assistant, Washington University.
Kasserman, Walter H., Washington and Jefferson College.
Norman, George R., Student, Wabash College.
Ormsbee, Richard A., Graduate Assistant, Brown University.
Rathbun, Edith N., 88 Fosdyke Street, Providence, Rhode Island.
Scholander, Per Fredrik, Research Associate, University of Oslo.
Stokes, Allen W., Harvard University.
Wolf, Mary H., Student, Duke University.
Woodward, Arthur, Jr., Graduate Assistant, Wesleyan University.

PROTOZOÖLOGY

Beam, Carl A., Student, Brown University.
Carroll, Kenneth M., Student, Franklin and Marshall College.
Cosgrove, William B., Student, Cornell University.
Dodge, Frances, Student, Gettysburg College.
Harrigan, Mary K., Special Instructor in Biology, Simmons College.
Macdonald, Katherine C., Graduate Student, McGill University.
Marchand, Doris, Teacher, St. Catherine's School, Richmond, Virginia.

INVERTEBRATE ZOÖLOGY

Adams, Esther F., Instructor in Biology, Moberly Junior College.
Allen, Jean, Miami University.
Beeman, Elizabeth A., Graduate Assistant in Zoölogy, Mount Holyoke College.
Bergstrom, William H., Student, Amherst College.

Böving, Bent G., Assistant, Swarthmore College.
Brush, Helen V., Assistant in Zoölogy, Vassar College.
Burns, John E., Graduate Laboratory Assistant, Wesleyan University.
Cairns, Malcolm G., New Jersey State Teachers College, Montclair, New Jersey.
Clark, Arnold M., Student, University of Pennsylvania.
Coe, Grace L., Student, New Jersey State Teachers College, Montclair, New Jersey.
Dent, James N., Graduate Assistant in Zoölogy, Johns Hopkins University.
Edwards, Gene C., Student, Wabash College.
Fitzgerald, Laurence R., State University of Iowa.
Gibbs, Elizabeth, Undergraduate, Wheaton College.
Goodrich, Mary W., Student, Wheaton College.
Gravett, Howard T., Associate Professor of Biology, Elon College.
Hale, Barbara, Student, Radcliffe College.
Hildebrandt, Wallace H., Undergraduate Instructor, Canisius College.
Holdsworth, Robert P., Jr., Austin Teaching Fellow, Harvard University.
Horwitz, Diana C., Teacher, Hyde Park High School, Hyde Park, Massachusetts.
Hoyt, Jane M., Barnard College.
James, Marian F., Graduate Fellow, University of Illinois.
Killough, John H., Graduate Student, Johns Hopkins University.
Kline, Irene T., Duke University.
Kreeger, Florence Brooks, Graduate Assistant in Biology, Newcomb College.
Lamoreux, Welford F., Assistant Professor, Cornell University.
Lerner, Eleanor, Brooklyn College.
Levitzky, Edward, Student, Rutgers University.
MacRae, Roberta M., Graduate Assistant, Wellesley College.
McKenzie, Helen E., Seton Hill College.
Marbarger, John P., Graduate Student, Johns Hopkins University.
Means, Oliver W., Jr., Yale University.
Micklewright, Helen L., Student, Wilson College.
Musser, Ruth E., Student, Goucher College.
Noce, Mildred W., Student, Southwestern College.
Powers, Samuel R., Jr., Swarthmore College.
Putnam, William S., Graduate Assistant, Amherst College.
Reeves, Walter P., Jr., Graduate Student, University of Alabama.
Royle, Jane G., Graduate Assistant in Anatomy and Invertebrate Zoölogy, Bryn Mawr College.
Samuels, Robert, University of Pennsylvania.
Saunders, Grace S., Hunter College.
Schnabel, Margaret J., Student, Oberlin College.
Scott, George T., Assistant, Harvard University.
Shank, Margaret L., Student, New Jersey State Teachers College, Montclair, New Jersey.
Smith, Fern W., Student, Smith College.
Smith, Frederick E., Massachusetts State College.
Smith, Julia P., Student, University of Rochester.
Stifler, Margaret C., Assistant, Goucher College.
Stone, Fred L., University of Rochester.
Syner, James C., Student, Springfield College.
Walker, Warren F., Jr., Student, Harvard University.
Wheeler, Bernice M., Instructor, Westbrook Junior College.
White, Francis M., Graduate Assistant in Biology, Purdue University.
Wolaver, John H., Jr., Student, DePauw University.
Wright, Margaret R., Student, Yale University.

4. TABULAR VIEW OF ATTENDANCE, 1936–1940

	1936	1937	1938	1939	1940
Investigators—Total	359	391	380	352	386
Independent	226	256	246	213	253
Under Instruction	76	74	53	60	62
Research Assistants	57	61	81	79	71
Students—Total	138	133	132	133	128
Zoölogy	55	57	54	55	55
Protozoölogy	17	16	10	12	7
Embryology	34	35	34	36	34
Physiology	22	16	22	21	22
Botany	10	9	12	9	10
Total Attendance	497	524	512	485	514
Less Persons registered as both students and investigators	24	13	16	14	7
	473	511	496	471	507
Institutions Represented—Total	158	165	151	162	148
By Investigators	120	134	125	132	112
By Students	77	79	67	72	79
Schools and Academies Represented					
By Investigators	2	3	4	2	1
By Students	3	2	1	2	2
Foreign Institutions Represented					
By Investigators	9	16	14	8	2
By Students	5	—	3	1	1

5. SUBSCRIBING AND COÖPERATING INSTITUTIONS

1940

Amherst College
Barnard College
Biological Institute, Philadelphia, Pennsylvania
Bowdoin College
Brooklyn College
Brown University
Bryn Mawr College
Canisius College
College of Physicians and Surgeons
Columbia University
Cornell University
Cornell University Medical College
DePauw University
Duke University
Fisk University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly and Company
Long Island University
Massachusetts State College
Memorial Hospital, New York City
Mount Holyoke College
Mount Sinai Hospital, New York City
Newcomb College
New York State Department of Health
New York University
New York University College of Medicine
New York University Washington Square College
North Carolina College for Negroes
Northwestern University
Oberlin College
Ohio State University
Princeton University
Purdue University
Radcliffe College
Rockefeller Foundation

Rockefeller Institute for Medical Research
Russell Sage College
Rutgers University
Smith College
Springfield College
Stanford University
State University of Iowa
Syracuse University
Syracuse University College of Medicine
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Kansas
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Pittsburgh
University of Rochester
University of Virginia
Vanderbilt University Medical School
Vassar College
Villanova College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Wheaton College
Wilson College
Yale University
Yale University Medical School

6. EVENING LECTURES, 1940

Friday, July 5
Dr. Leonor Michaelis ..... "Oxidation and Reduction in Organic and Biological Chemistry."

Friday, July 12
Dr. Kenneth V. Thimann ..... "Hormones and the Physiology of Growth in Plants."

Friday, July 19
Dr. K. S. Cole ..... "Electrical Properties of the Cell Membrane."

Friday, July 26
Dr. D. H. Wenrich ..... "Chromosomes in Protozoa."

Wednesday, July 31
Mr. George C. Lower ..... "Local Marine Life in Color."

Friday, August 2
Dr. Eric G. Ball ..... "Catalysts of Biological Oxidations, their Composition and Mode of Action."

Thursday, August 8
Dr. L. J. Milne ..... "Animated Diagrams of Biological Processes."

Friday, August 9
Prof. F. O. Schmitt ..... "Modern Concepts of Protoplasmic Organization."

Friday, August 16
Dr. Alfred S. Romer ..... "The Phylogeny and Structure of the Lower Vertebrates."

Friday, August 23
Dr. Dugald Brown ..... "The Regulation of Metabolism in Contracting Muscle."

Friday, August 30

Dr. Curt Stern ..... "On Dependent Growth and Form of the Testes in Various Species of Drosophila."

Saturday, August 31

Dr. Per Höst ..... "Arctic Seal Hunting in the White Sea and in Greenland Waters."

7. SHORTER SCIENTIFIC PAPERS, 1940

Tuesday, July 9

Mr. Edward L. Chambers ..... "Inter-relations between Egg-Nucleus, Sperm-Nucleus and Cytoplasm of the Asterias Egg."

Dr. Daniel Mazia ..... "Digestion Studies on Salivary Chromosomes."

Dr. M. J. Kopac ..... "Some Properties of the Residue from Rapidly Disintegrated Arbacia Egg Cytoplasm."

Tuesday, July 16

Dr. S. C. Brooks ..... "Ion Intake by Living Cells."

Dr. L. I. Katzin ..... "The Use of Radioactive Tracers in the Determination of Irreciprocal Permeability of Biological Membranes."

Dr. K. C. Fisher ..... "Urethane and the Respiration of Yeast Cells."

Dr. M. M. Brooks ..... "Spectrophotometric Determinations on Hemoglobin and its Derivatives."

Tuesday, July 23

Dr. Nelson J. Spratt, Jr. ..... "An in vitro Analysis of the Organization of the Eye-forming Area in the Early Chick Blastoderm."

Dr. Ernest Scharrer ..... "On the Determination of the Vascular Pattern of the Brain of the Opposum."

Dr. Paul Weiss ..... "Functional Properties of Transplanted and Deranged Parts of the Central Nervous System of Amphibians."

Tuesday, July 30

Dr. B. H. Willier ..... "A Study of Feather Color Patterns Produced by Grafting Melanophores During Embryonic Development."

Dr. H. B. Goodrich ..... "The Cellular Basis of the Color Pattern in some Bermuda Coral Reef Fish."

Tuesday, August 6

DR. ALBERT E. OXFORD ..... "Production of a Complex Nitrogenous Compound Related to Tyrosine by a Species of Penicillium."

DR. KURT SALOMON ..... "Studies on Erythrocruorin (Invertebrate Hemoglobin)."

DR. KURT G. STERN,
DR. JOSEPH L. MELNICK AND
DR. DELAFIELD DUBOIS ..... "Photochemical Spectrum of the Pasteur Enzyme."

Tuesday, August 13

DR. A. C. GIESE ..... "Effects of Ultra-violet Light on Respiration of the Luminous Bacteria."

DR. IVOR CORNMAN ..... "Effects of Ether upon the Development of Drosophila melanogaster."

DR. BERTA SCHARRER ..... "Neurosecretory Cells in Cockroaches."

DR. G. HAUGAARD ..... "The Mechanism of the Glass Electrode."

Tuesday, August 20

DR. W. GORDON WHALEY ..... "Developmental Changes in Apical Meristems."

DR. HARRY G. ALBAUM AND
DR. BARRY COMMONER ..... "The Relation between the Four-Carbon Acid Respiratory System and the Growth of Oat Seedlings."

DR. R. K. SKOW AND
L. R. BLINKS ..... "Respiratory Changes following Stimulation in Nitella."

DR. L. R. BLINKS ..... "The Relation of Potassium to the Bio-electric Effects of Temperature and Light in Valonia."

8. GENERAL SCIENTIFIC MEETINGS, 1940

Tuesday, August 27

DR. S. O. MAST AND
DR. W. J. BOWEN ..... "The Hydrogen Ion and the Osmotic Concentrations of the Cytoplasm in Vorticella Similis Stokes, as Indicated by Observations on the Food Vacuoles."

DR. M. H. JACOBS AND
MR. W. D. JONES ..... "The Reversibility of Certain Artificially Induced Changes in the Permeability of the Erythrocyte."

Dr. E. J. Boell,
Dr. R. Chambers,
Miss E. A. Glancy,
Dr. K. G. Stern and
Miss B. Meyerhof ..... "Oxidase Activity and Respiration of Cells and Cell Fragments."

Dr. E. J. Boell and
Dr. L. L. Woodruff ..... "Respiratory Metabolism of Mating Types in Paramecium Calkinsi."

Dr. Eric G. Ball and
Miss Pauline A. Ramsdell ..... "Squid Ink, a Study of its Composition and Enzymatic Production."

Dr. A. E. Oxford ..... "Observations on the Occurrence of Simple Ethereal Sulphates in Marine Algae."

Dr. E. J. W. Barrington ..... "Blood-sugar and the Problem of the Pancreas in Lampreys."

Dr. A. E. Navez and
Mr. A. DuBois ..... "Fatty Acid Compounds in the Unfertilized Egg of Arbacia punctulata."

Mr. C. B. Giddings ..... "Distribution of Plasmalogen in Certain Invertebrate Forms."

Dr. G. H. Parker ..... "Lipoids and their probable Relation to Melanophore Activity."

Mr. Samuel Belfer,
Mr. B. Bailey,
Dr. H. C. Bradley, and
Mr. Howard Eder ..... "Studies of the Distribution of the Autolytic Mechanism."

Dr. Carl C. Smith ..... "The Effect of Various Cholinergic Drugs on the Radula Protractor Muscle of Busycon canaliculatum."

Dr. E. J. Boell and
Dr. D. Nachmansohn ..... "Choline Esterase in Nerve Fibers."

Dr. R. G. Abell and
Dr. Irvine H. Page ..... "Vascular Reactions to Renin and Angiotonin."

Mr. J. Crawford,
Miss D. Benedict, and
Dr. A. E. Navez ..... "Factors Affecting the Frequency of Contraction of the Heart of Venus mercenaria."

Mr. Charles E. Wilde, Jr. ..... "Determining Factors in the Regeneration of Hydractinia."

Dr. Edgar Zwilling ..... "Time of Determination and Dominance in Tubularian Reconstitution."

Dr. S. Meryl Rose ..... "A Regeneration-Inhibiting Substance Released by Tubularia Tissue."

Dr. L. G. Barth ..... "The Rôle of Oxygen in Regeneration of Tubularia."

Dr. Harry G. Albaum ..... "The Growth of Oat Coleoptiles after Seed Exposure to Different Oxygen Concentrations."

Dr. W. Gardner Lynn ..... "Results of Transplantation of the Pituitary Anlage to the Thyroid Region in Amblystoma."

Wednesday, August 28

Dr. T. C. Evans ..... "Oxygen Consumption of Arbacia Eggs Following Exposure to Roentgen Radiation."

Dr. T. C. Evans ..... "Effects of Roentgen Radiation on the Jelly of Arbacia Egg. I. Disintegration of the Jelly."

Dr. M. E. Smith and Dr. T. C. Evans ..... "Effects of Roentgen Radiation on the Jelly of the Arbacia Egg. II. Changes in pH of Egg Media."

Mr. E. P. Little and Dr. T. C. Evans ..... "Delay in First Cleavage of Arbacia Eggs Following Roentgen Irradiation of Zygotes."

Dr. Grace Townsend ..... "Concerning Sensitivity of Cells to X-Ray."

Dr. Grace Townsend ..... "Laboratory Ripening of Arbacia in Winter."

Dr. Ethel Browne Harvey ..I. "A Note on Determining the Sex of Arbacia punctulata."

Dr. Ethel Browne Harvey .II. "Centrifugal Speed and the Arbacia Egg."

Dr. Ethel Browne Harvey III. "Colored Photographs of Stratified Arbacia punctulata Eggs Stained with Vital Dyes."

Dr. Herbert Shapiro ..... "Elongation and Return in Spherical Cells."

Mr. Ivor Cornman ..... "Echinochrome as the Sperm-activating Agent in Egg-water."

Mr. Teru Hayashi ..... "A Relation between the Dilution Medium and the Survival of Spermatozoa of Arbacia punctulata."

Dr. Wm. H. F. Addison ..... "The Occurrence of Cartilage at the Bifurcation of the Common Carotid Artery in an Adult Dog."

Dr. Hope Hibbard ..... "Cytoplasmic Morphology in the Gizzard of Gallus domesticus."

PAPERS READ BY TITLE

MR. FRED W. ALSUP ..... "Further Studies of Photodynamic Action in the Eggs of Nereis limbata."

MR. C. W. J. ARMSTRONG AND
DR. KENNETH C. FISHER ..... "A Quantitative Study of the Effect of Cyanide and Azide on Carbonic Anhydrase."

DR. FRANK A. BROWN, JR., AND
DR. ALISON MEGLITSCH ..... "Upon the Sources in the Insect Head of Substances which Influence Crustacean Chromatophores."

DR. RALPH H. CHENEY ..... "Myofibrillar Modifications in the Caffeinized Frog Heart."

DR. LEONARD B. CLARK ..... "Effects of Visible Radiation on Arbacia Eggs Sensitized with Rhodamine B."

DR. A. C. CLEMENT ..... "Effects of Cyanide on Cleavage in Eggs of Ilyanassa and Crepidula."

DR. D. P. COSTELLO ..... "The Cell Origin of the Prototroch of Nereis limbata."

DR. JAMES DONNELLON ..... "Blood Clotting in Callinectes sapidus."

DR. LLEWELLYN T. EVANS ..... "Effects of Light and Hormones upon the Activity of Young Turtles, Chrysemys picta."

DR. LLEWELLYN T. EVANS ..... "Effects of Testosterone Propionate upon Social Dominance in Young Turtles, Chrysemys picta."

DR. KENNETH C. FISHER AND
MR. RICHARD J. HENRY ..... "The Use of Urethane as an Indicator of "Activity" Metabolism in the Sea Urchin Egg."

MR. MORDECAI L. GABRIEL ..... "The Inflation Mechanism of Spheroides maculatus."

MISS E. A. GLANCY ..... "Micromanipulative Studies on the Nuclear Matrix of Chironomus Salivary Glands."

DR. JOHN E. HARRIS ..... "The Reversible Nature of the Potassium Loss from Erythrocytes during Storage of Blood at 2–5° C."

DR. ARNE V. HUNNINEN AND
DR. RAYMOND M. CABLE ..... "Studies on the Life History of Anisoporus Manteri sp. nov. (Trematoda: Allocreadiidae)."

DR. CORNELIUS T. KAYLOR ..... "Histological Studies on the Problem of Edema in Haploid Triturus pyrrhogaster Larvae."

Dr. Balduin Lucké,
Dr. Arthur K. Parpart, and
Mr. R. A. Ricca ..... "Do Carcinogenic Compounds affect Cell Permeability?"

Dr. W. G. Lynn ..... "The Development of the Skull in the Non-aquatic Larva of the Tree-toad, Eleutherodactylus nubicola."

Dr. W. G. Lynn ..... "The Embryonic Origin and Development of the Pharyngeal Derivatives in Eleutherodactylus nubicola."

Sister Maria Laurence Maher . "Preliminary Report on Effect of Indole Acetic Acid on Growth of Chlamydomonas."

Dr. H. Shapiro ..... "Further Studies on the Metabolism of Cell Fragments."

Dr. Carl C. Smith,
Miss Blanche Jackson and
Dr. C. Ladd Prosser ..... "Responses to Acetylcholine and Cholinesterase Content of Cerebratulus."

Dr. A. J. Waterman ..... "Response of the Heart of the Compound Ascidian, Perophora Viridis, to Pilocarpine, Atropine and Nicotine."

DEMONSTRATIONS

Wednesday, August 28

Dr. W. H. F. Addison ..... "Corrosion Preparations of the Branchial Circulation in the Dogfish."

Dr. E. Scharrer ..... "Vascularization of the Extramedullary Nerve Cells of the Puffer, Spheroides Maculatus."

Dr. E. R. Clark and
Mrs. Eleanor Linton Clark .... "The Microscopic Study of Living Tissues in Transparent Chambers Installed in Rabbits' Ears."

Mr. E. P. Little ..... "Color and Luminescence Produced by Roentgen Rays in Glass and Chemicals."

Dr. E. J. Boell ..... "The Cartesian Diver Ultramicro-Respirometer."

Per F. Scholander,
Dr. S. W. Grinnell and
Dr. L. Irving ..... "Apparatus for Measurement of Respiratory Metabolism and Circulation Changes."

9. MEMBERS OF THE CORPORATION, 1940

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
King, Mr. Chas. A.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Lowell, Mr. A. L., 17 Quincy Street, Cambridge, Massachusetts.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Streets, New York City, New York.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Morgan, Mrs. T. H., Pasadena, California.
Morrill, Dr. A. D., Hamilton College, Clinton, New York.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, New York.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, New York.
Trelease, Prof. William, University of Illinois, Urbana, Illinois.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Abramowitz, Dr. Alexander A., Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.

ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.

ALBAUM, DR. HARRY G., 3115 Avenue I, Brooklyn, New York.

ALLEE, DR. W. C., The University of Chicago, Chicago, Illinois.

AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

ANDERSON, DR. RUBERT S., Memorial Hospital, 444 East 58th Street, New York City, New York.

ANGERER, DR. CLIFFORD A., Department of Physiology, Ohio State University, Cleveland, Ohio.

ARMSTRONG, DR. PHILIP B., College of Medicine, Syracuse University, Syracuse, New York.

AUSTIN, DR. MARY L., Wellesley College, Wellesley, Massachusetts.

BAITSELL, DR. GEORGE A., Yale University, New Haven, Connecticut.

BAKER, DR. H. B., Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

BALLARD, DR. WILLIAM W., Dartmouth College, Hanover, New Hampshire.

BALL, DR. ERIC G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

BARD, PROF. PHILIP, Johns Hopkins Medical School, Baltimore, Maryland.

BARRON, DR. E. S. GUZMAN, Department of Medicine, The University of Chicago, Chicago, Illinois.

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DECOMPOSITION AND REGENERATION OF NITROGENOUS ORGANIC MATTER IN SEA WATER

IV. Interrelationship of Various Stages; Influence of Concentration and Nature of Particulate Matter [1]

THEODOR VON BRAND AND NORRIS W. RAKESTRAW

(From the Woods Hole Oceanographic Institution, Woods Hole, Mass.)

In previous investigations (1937, 1939, 1940) it has been demonstrated that the decomposition of particulate organic matter in sea water proceeds in well-defined steps, the main stages being the formation of ammonia, nitrite and nitrate. The appearance of these substances is due to the successive development of different bacterial floras acting upon the original substratum of organic matter. In nature, however, a mixture of the various floras will probably occur, with frequent or continuous addition of new decomposing material. It seemed desirable, therefore, to study the interrelationship of the different stages of the cycle and the results obtained when two or more stages occur simultaneously.

For this purpose a 20-liter carboy of filtered sea water from Woods Hole Harbor, to which washed diatoms (*Nitzschia Closterium*) were added, was kept at room temperature in the dark. In order to determine what effect the bacterial flora present at various stages would have on the decomposition of fresh organic matter, portions of the culture were withdrawn at various times during the decomposition cycle. New particulate organic matter was added to these subcultures, as indicated below, after which they were put in fresh containers in the dark. The parent culture (No. 42) and the various subcultures (42A to 42H) were analyzed regularly for the different forms of nitrogen and the changes found are shown in Fig. 1.

The first subculture, No. 42A, was separated from the parent culture when the ammonia in the latter had reached its maximum and when the first trace of nitrite had appeared; the second subculture (42B) a few days later, when the nitrite formation was well under way; and the third (42C), when the nitrite had about reached its maximum. Later, when the nitrite began to decline in subculture No. 42B and when nitrate formation had begun, a portion of it was used in preparing a new subculture (42G). To all these subcultures new particulate matter was added in the form of living, washed diatoms.

[1] Contribution No. 292 from the Woods Hole Oceanographic Institution.

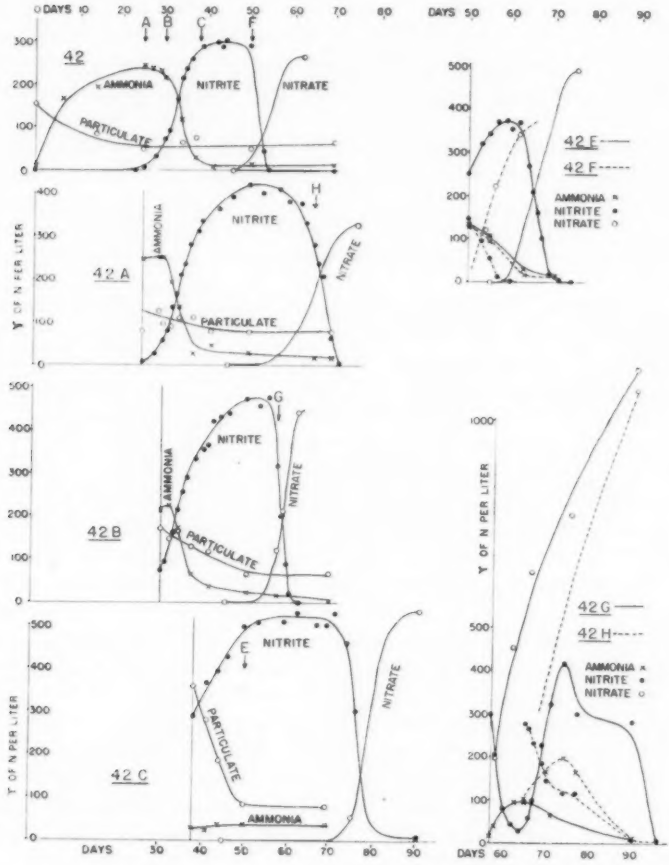

FIG. 1. Interrelationship of different stages of the decomposition cycle. Time in days. Different forms of nitrogen in micrograms (gamma) per liter. The original culture, No. 42, consisted of filtered sea water with washed diatoms (*Nitzschia Closterium*) added. Decomposition in the dark. Subcultures *A*, *B*, etc., separated at times indicated by arrows and with new particulate organic matter added.

Figure 1 shows that nitrite formation in the first three subcultures (42A, B and C) was in no way interfered with by the addition of new diatoms, but proceeded at normal speed without interruption. In each case ammonia rose only slightly higher than in the original culture, No.

42, indicating that the ammonia formed from the new decomposing diatoms was at once oxidized to nitrite. Finally, the nitrite disappeared from all cultures in the usual way, appearing quantitatively as nitrate.

A somewhat different result was obtained in subculture 42G, which was prepared by adding new organic matter to a portion of 42B when the latter was approaching the end of the nitrite stage. As before, ammonia remained low throughout the whole time. During the first week nitrite disappeared exactly as in the mother-culture from which it had been prepared (42B), but after this it increased again, reaching a new maximum ten days later. Apparently the nitrite-forming flora was on the decline when this subculture was begun but was able to recover under the influence of the newly-formed products of decomposition. Nitrate formation seems to have occurred throughout this subseries. It is probable that we have here a case in which ammonia-, nitrite- and nitrate-forming floras were active at the same time.

In the cultures so far described living diatoms were used as a source of new organic matter; consequently, vigorous ammonia formation was not actually under way at the start of each subculture. In the next two cultures organic matter was introduced which was already in the ammonia-formation stage. Fresh diatoms were added to a fresh quantity of harbor water (No. 42D). After 12 days in the dark, when ammonia was being formed rapidly, portions of this culture were withdrawn and mixed with equal amounts of older cultures in various stages of the decomposition cycle. Thus, subculture 42E consisted of an equal mixture of 42D and 42C, the latter taken when the nitrite had reached its maximum. In this case the ammonia introduced with culture 42D disappeared rapidly, with a corresponding rise in nitrite. Evidently the nitrite-forming flora of culture 42C was still active when the new, partially-decomposed organic matter was added.

Subculture 42F was prepared by separating a portion of the original culture, No. 42, when nitrite had begun to diminish, and adding an equal amount of 42D containing organic matter in the stage of ammonia formation. Both ammonia and nitrite disappeared rapidly, in contrast to the last preceding subculture, 42E, probably due to the fact that the nitrate-forming flora in the parent culture was at that time the most potent one.

The last culture, 42H, behaved in a somewhat similar manner. This consisted of a portion of culture 42A, separated at a time when the nitrite was about half converted to nitrate. To this was added a large number of partially decomposed diatoms, centrifuged from a culture which had stood for six days in the dark. A relatively small increase in ammonia was observed during the first days, with a subsequent rapid decrease.

Nitrite was present somewhat longer than in 42A, but never reached a very high level. It is likely that during this whole time nitrate formation proceeded rapidly.

The following conclusions may be drawn from the study of culture 42 and its subcultures: Ammonia formation does not interfere with the formation of either nitrite or nitrate, in such concentrations as we observed. The strict sequence of processes in our normal decomposition experiments can therefore hardly be due to any inhibiting action of ammonia or other initial products of decomposition upon nitrite or nitrate formation. More likely is it connected with a very slow development of the oxidizing floras. Doubtless, however, some other, hitherto unrecognized factor must also be involved. A slow increase in the nitrate-forming population, for example, is alone insufficient to explain why it should require weeks, or even months, for the first traces of nitrate to appear, whereas once the process has started the nitrate maximum may be reached in five days.

These observations are not necessarily inconsistent with experiments we have described previously, involving deep sea water, in which there was evidence of a retarding influence on the development of the oxidizing floras. This influence has not yet been explained, but seems to be connected with some unknown special property of the deep sea water used.

The course which the decomposition will take, when new organic material is added, will depend upon the flora which predominates. In general, a shortening of the cycle will occur, as far as the newly added material is concerned. The original culture, No. 42, took 55 days to complete its cycle. In the various subcultures the mean time from the addition of new organic matter to the end of the cycle was 36 days, or 41 days if one includes the initial period of decomposition of the added organic matter before its addition to Series 42E, F and H.

Duration of the Cycle

As pointed out in previous papers, the time required for the decomposition cycle varies considerably in different series. It seemed possible that the initial concentration of organic matter might be a determining factor in this connection and Series 47 to 50 were set up to investigate this point. The four cultures contained amounts of particulate nitrogen varying from 185 γ to 768 γ per liter. As shown in Fig. 2, this factor seems to be of some, though not of very great importance. The rate of disappearance of particulate nitrogen was nearly the same in each case. In the higher concentrations the ammonia maxi-

mum was reached a little earlier and nitrite appeared and disappeared more rapidly. The total time for the cycle varied from 61 days in the highest concentration to 88 days in the lowest.

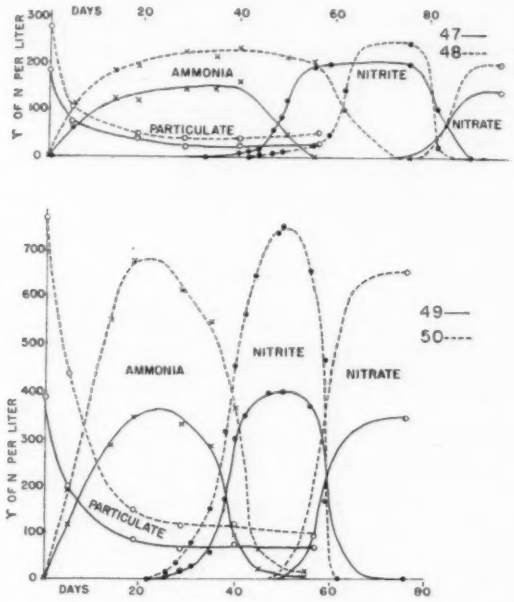

FIG. 2. Series 47 to 50. Influence of varying concentration of particulate matter. Filtered sea water with different amounts of *Nitzschia Closterium* added. Decomposition in the dark. Time in days. Different forms of nitrogen in micrograms (gamma) per liter.

The nature of the suspended organic matter also determines the duration of the cycle, as the next series show. Series 43 and 45 (Figs. 3 and 4) were set up with the same harbor water; in 43 was suspended a small amount of mixed plankton, in 45 a large amount of yeast. In No. 43 the nitrogen cycle proceeded in the normal way, but in No. 45 nitrite appeared only very slowly, with no formation of nitrate when the experiment was terminated after 5½ months.

It has been shown that the length of the decomposition cycle depends upon the source of the water and it has been suggested that this might involve the action of growth-promoting substances upon the bacterial flora. Series 44 and 46 (Figs. 3 and 4) were planned as an approach to this question. Two samples of harbor water, the same as in Nos. 43

and 45, were evaporated to dryness and the salt residues ignited at 600–700° C. for 5 hours, to destroy organic matter. The salts were dissolved in the original volume of distilled water, with a little HCl, and the pH brought back to between 7.5 and 8.2 with NaOH. To the two (Nos. 44 and 46) were added amounts of mixed plankton and yeast, respectively, corresponding to the quantities in Series 43 and 45. In

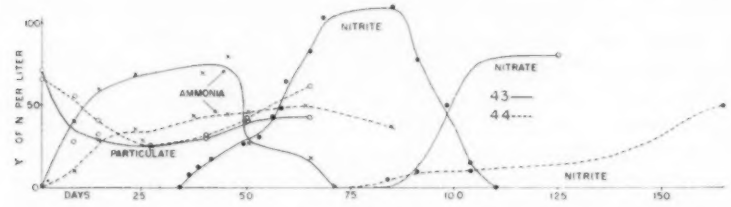

FIG. 3. Series 43 and 44. Mixed plankton added to filtered sea water (No. 43) and to a "synthetic" water made by redissolving the ignited salt residue of evaporated sea water (No. 44). Time in days. Different forms of nitrogen in micrograms (gamma) per liter.

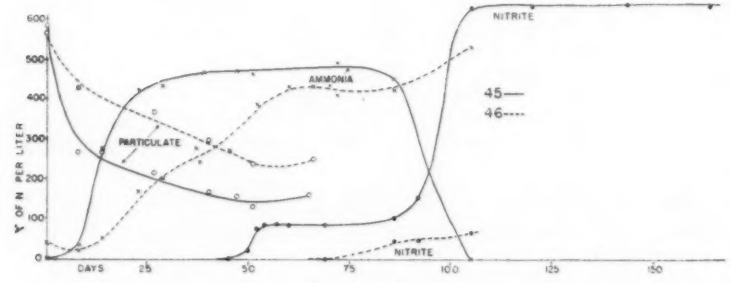

FIG. 4. Series 45 and 46. Yeast added to filtered sea water (No. 45) and to a "synthetic" water made by redissolving the ignited salt residue of evaporated sea water (No. 46). Time in days. Different forms of nitrogen in micrograms (gamma) per liter.

both 44 and 46 the formation of ammonia was much slower than in the untreated water of Series 43 and 45, an effect even more pronounced on the formation of nitrite, which had not reached its maximum at the termination of the experiments. This apparently indicates that some "growth-promoting factor" had been eliminated from the water by the process of ignition, but further investigation will be necessary before a definite conclusion can be reached.

SUMMARY

1. With a recurrent supply of particulate organic matter, the formation of ammonia, nitrite and nitrate may take place simultaneously. The process which predominates will depend upon the stage at which the new organic matter is introduced.

2. The nature of the suspended particulate matter is of considerable importance in determining the total duration of the decomposition cycle, but the level of its original concentration is only a minor determining factor.

3. There is some evidence of a "growth-promoting" factor, normally effective in the decomposition cycle, but which can be destroyed by high temperature.

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THE REPRODUCTIVE CYCLE OF THE VIVIPAROUS TELEOST, NEOTOCA BILINEATA, A MEMBER OF THE FAMILY GOODEIDAE

III. The Germ Cell Cycle

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Introduction

In previous articles on the reproductive cycle of *Neotoca bilineata*, the writer has described the breeding cycle and the somatic cycle of the ovary (Mendoza, 1939, 1940). It was shown that, in *Neotoca*, as is true for many viviparous teleosts, the height of the breeding season occurs during the spring and early summer; broods are spaced approximately 44 days apart. During the breeding cycle, the soma of the ovary was shown to undergo very marked cyclic changes. These changes include (*a*) an increased tumescence of the ovarian stroma, (*b*) a secretory activation of the internal ovarian epithelium, and (*c*) marked changes in the free cellular elements. These conditions are particularly prominent during the middle stages of gestation and later recede to the more normal resting condition. In order to complete the analysis of the reproductive cycle, a study was made of the germ cells and their cyclic variation during gestation. The present account, therefore, presents such an analysis of the germ cells. The detailed description of the germ cells, follicles, their changes during growth, and the fate of the atretic and evacuated follicles, etc., will be considered in a later report.

Previous accounts of the reproductive cycle of viviparous teleosts have made little or no reference to the germ cell cycle, an omission that is difficult to justify since, obviously, the phenomenon is an important phase of the reproductive cycle. It is true, of course, that in many investigations of viviparous teleosts the paucity of material prevented a detailed analysis of the complete gestation cycle. Among recent accounts, the ones on *Jenynsia* (*Fitzroyia*) *lineata*[1] (Scott, 1928) and *Xiphophorus helleri* (Bailey, 1933) contain no reference to the germ cell cycle whereas

[1] E. J. MacDonagh of the Museo de La Plata has kindly informed me that the name *Jenynsia lineata* is considered preferable over the name *Fitzroyia lineata*. In two publications (1934, 1938), MacDonagh uses the name *Jenynsia;* in the latter, he quotes correspondence with C. L. Hubbs of the University of Michigan in which the latter supports the use of the name *Jenynsia lineata*.

Turner (1938*a*) mentions it but briefly in his description of the reproductive cycle of *Cymatogaster aggregatus*. However, Turner does devote considerable attention to the germ cells in his general study of poeciliid fishes (Turner, 1937) and in a special article on the poeciliid, *Brachyrhaphis episcopi* (Turner, 1938*b*). In general, nevertheless, a complete count and measurement of the germ cells during gestation has not been made in a viviparous teleost. Hence it is the purpose of the present account to supplement a preliminary description (Mendoza, 1938) by considering in greater detail the cyclic variation of the germ cells during gestation.

Materials

The present analysis of the germ cell cycle is based on a study of the gonads used in the preceding study on the somatic cycle. Fourteen of these ovaries were chosen at well-spaced intervals during the resting and gestation periods. Detailed cell counts were made in one lateral half of each of the above ovaries; these cell counts, involving a total of 4686 oöcytes, form the basis of the present analysis. The brief description of the germ cells and follicles is based on gonads fixed in Bouin's fluid and stained with iron hematoxylin. In a considerable number of sections, Mallory's triple connective tissue stain was used. A detailed account of the ovaries used and the method of treating the data is given later.

Description of the Germ Cells

As is true for all the Goodeidae, the germinal tissue is confined to the lobulated, ovigerous folds in the ovary. In *Neotoca* there are two such folds, one on either side of the median sagittal septum. The germ cells are confined solely to the ovigerous folds and are more or less evenly distributed throughout the gonad except at the extreme anterior and posterior ends.

In general, the oöcytes of *Neotoca* are essentially similar to those of other viviparous teleosts. The eggs are spherical, attain a maximal size of 180–200 micra in diameter and are characterized by the absence of large masses of yolk. The cytoplasm is coarsely granular and contains the scattered, flocculent, albumen-like yolk. The nucleus of the fully-grown oöcyte is granular, oxyphylic in reaction, and contains typical "lampbrush" chromosomes. Numerous vacuolated nucleoli may appear within the nucleus. Surounding the oöcyte is a follicle composed of a single row of tall columnar cells tightly pressed together. External to the follicle is a thin sheath of connective tissue fibers in which appears a plexus of capillaries.

Germ Cell Cycle

A detailed account of the germ cell cycle during gestation was obtained by using fourteen females (see Table I), chosen at well-spaced intervals before and during gestation. In each female a count was made of every oöcyte, germ cell nest, and atretic follicle in one of the two ovigerous folds. Each oöcyte counted was measured and placed in one of six groups depending upon its diameter (see Table II). The groups

Table I

Females used in the analysis of the germ cell cycle.

Number of Female	Stage of Gestation	Number of Female	Stage of Gestation
6	Non-gravid ovary	12	Embryos 4.5 mm. in length
20	Non-gravid ovary	8	" 4.5 " " "
22	Early segmentation	23	" 6.0 " " "
19	Late segmentation	27	" 6.0 " " "
47	Embryos 1.5 mm. in length	9	" 7.0 " " "
16	" 2.7 " " "	11	" 7.2 " " "
44	" 3.5 " " "	1	" 9.0 " " "

were chosen arbitrarily to facilitate counting and measuring the cells; the actual limits of each group were determined largely by the ocular micrometer units at that particular magnification. Since the total number of oöcytes in each group would vary with the size of the gonad, the average number of cells per half-section of ovary was obtained. Thus, an average figure was obtained that could be compared with those of other gonads regardless of size differences. In order to further reduce the possibilities of individual vaiations of different ovaries, counts were made, wherever possible, of two ovaries for each stage of gestation. Thus six of the eight representative stages chosen are based on the average figures between two different ovaries; only in two stages, IV (3.5 mm.) and VIII (9.0 mm.) are the figures based on a single ovary. All in all, fourteen ovaries were examined histologically, 5633 sections were checked for oöcytes, and 4686 germ cells were counted and measured (see Table II). Graph I is based on these figures. From a careful study of these graphs and figures certain definite and interesting conclusions were obtained.

Conclusions on the Germ Cell Cycle

Continuous Production of Oöcytes

There is no evidence of a complete cessation of egg production during gestation. Nests occur abundantly at all times although, with the excep-

TABLE II

The germ cell count during gestation.

In order to facilitate the analysis of the germ cells an arbitrary segregation was made of the oöcytes into germ cell nests and six other groups on the basis of size. The six groups and the diameter in micra of the oöcytes involved are indicated below. Similarly, gestation and the resting period were divided into eight arbitrary stages. The pre-fertilization or resting period forms Stage I; thereafter the different stages are distinguished by the stage of development or size of the contained young. Birth normally follows immediately after Stage VIII. In all Stages except IV and VIII, two ovaries were used for the analysis of the germ cells. By using two ovaries it was hoped to get a more typical picture. In the two exceptional cases only one ovary was available for each, but since both ovaries were normal in every respect it is assumed that the cell counts also are typical. For each cell group there are two figures: the whole numbers are the total numbers of cells of each particular group in the different stages of gestation; the numbers in decimals represent the average number of cells of each group per half-section of the ovary.

Number of Ovary	Stage of Gestation	Number of Sections	Number of Nests	Groups of Oöcytes					
				I 10–36μ	II 40–72μ	III 76–108μ	IV 112–144μ	V 148–180μ	VI 184–216μ
6, 20	I Resting	651	136 .2149	560 .8889	121 .1993	54 .0861	29 .0461	41 .0635	4 .0061
19, 22	II Segmentation	640	169 .2641	496 .7750	178 .2781	57 .0891	32 .0500	26 .0406	4 .0063
16, 47	III 2.0 mm.	437	83 .1899	253 .5789	79 .1878	39 .0892	26 .0595	14 .0320	
44	IV 3.5 mm.	154	29 .1883	86 .5584	29 .1883	14 .0909	8 .0521	6 .0389	
8, 12	V 4.5 mm.	507	129 .2544	245 .4832	92 .1815	33 .0651	23 .0454	12 .0237	
23, 27	VI 6.0 mm.	977	106 .1085	681 .6970	162 .1658	54 .0553	65 .0665	14 .0144	
9, 11	VII 7.2 mm.	1417	153 .1079	304 .2145	143 .1009	50 .0353	25 .0176	12 .0085	1 .0007
1	VIII 9.0 mm.	850	128 .1506	493 .5800	72 .0847	22 .0259	14 .0165	13 .0153	
	Totals	5633	933	3118	876	323	222	138	9

tion of the rise in number of nests in Stage V, it can be stated that nests of germ cells decrease in number until the latter part of gestation; at that time they are approximately half as abundant as in pre-fertilization stages.

Time of Onset of Egg-production

The onset of the wave of egg-production for the following brood is not a prominent one as is true for some of the poeciliids described by Turner (1937). Following the general decrease in number of nests and small oöcytes during gestation, there is a rather sharp rise again at the end of gestation when the current brood is about ready for birth. It is unlikely that these minute oöcytes are the ones destined for fertilization and the formation of the following brood. Rather it is more probable that the rise in number of small oöcytes is the first indication of a general activation which results in an increase in the number of eggs of all groups before the following fertilization period. Hence, it is more likely that eggs of Groups III and IV will grow sufficiently during the resting period to form the bulk of eggs to be fertilized for the succeeding brood. Growth of these larger eggs, however, must of necessity occur in the interval between the expulsion of the current brood and the fertilization of the next group of eggs since there is no indication of an increase in number among these larger groups (III and IV) before the end of gestation. The only other noticeable increase in the number of eggs before the end of gestation apparently occurs in Group V eggs, and that increase is but a slight one.

Variation in Number of Eggs with Size

Regardless of the stage of gestation, oöcytes of the 10–36 μ group are the most numerous. Following that there is a regular and almost perfect drop in the number of cells in each succeedingly larger group so that, actually, cells of maximal size, 184–216 μ, are the smallest in number. Only two exceptions occur to this generalization. The first, in Stage VI of gestation, is caused by an abnormally large count of cells of Group 112–144 μ in ovary number 27; this may be purely an individual variation. The second exception is one that is readily understood. In Stage I, preceding gestation, cells of Group 148–180 μ include the bulk of the cells that are to be fertilized and hence appear in large numbers. The number is so large, in fact, that it exceeds that of the next smaller group of cells, Group IV. Furthermore, it is interesting to note that, during the stage of segmentation, since a large percentage of the cells of Group V were fertilized, the count once more drops below that of Group IV eggs and remains so during the rest of gestation.

Decrease in Number of Eggs During Gestation

Not only do eggs decrease in number with increase in size in any one ovary but eggs of all sizes also decrease in number during gestation.

The cell count for each size group is maintained fairly well until the middle period of gestation; from that time on there is a noticeable drop in the number of cells to one-third or one-quarter of their number in the middle stages of gestation. In keeping with this observation, there is a noticeable increase in atretic follicles during the latter half of gestation.

Maximal Size of Eggs

Normally, oöcytes attain maximal size during the resting period of the ovary and remain until stages of segmentation. Following that there is a noticeable absence of large eggs. This condition is verified further by the large number of degenerating follicles of maximal size which are found in pre-gestation stages. Absence of large degenerating follicles and large eggs in the later stages of gestation indicates failure of eggs normally to attain maximal size during that period.

Superfetation

It has been determined before (Turner, 1933; Mendoza, 1939) that superfetation normally does not occur among the growing embryos. It is interesting that, in keeping with this fact, there is no evidence among growing oöcytes of a phenomenon similar to superfetation; rather there is a continuous gradation in the size of oöcytes in all ovaries.

Variation in Number of Oöcytes

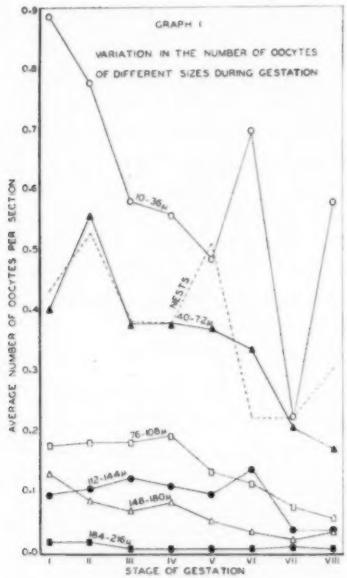
The fluctuation in number of oöcytes of different sizes during gestation varies with the size of the cells. Larger eggs vary but little in number during gestation; smaller oöcytes, however, fluctuate widely in number. The almost perfect inverse relation between the size of oöcytes and fluctuation in number is clearly evident in Table III.

Percentage of Eggs Fertilized

Failure to find fertilization occurring before the expulsion of the previous brood is explainable, in part at least, by the presence of only 20–25 per cent as many large eggs at the end of gestation as there are in the resting ovary immediately preceding the time for fertilization.

An indication of the large number of eggs prepared for fertilization is obtained from the fact that despite the large number of atretic follicles in the gonad prior to fertilization, there still is a greater number of large eggs present at that time than at any other stage of gestation. It appears likely that fully 40–50 per cent of the large eggs available at time of

fertilization actually are activated to start development. This is verified by the knowledge that a normal brood averages from fifteen to twenty young, a number which is approximately half of the total number of



EXPLANATION OF GRAPH I

The graph represents in diagrammatic form the variation of germ cells during gestation. Each curve represents the number of cells of a limited size range during each of the stages of gestation. The different curves are identified by the diameters of the eggs involved. The group of the smallest oöcytes is called Group I; groups of successively larger eggs are called respectively Groups II, III, etc. The stages of gestation are plotted on the horizontal axis (see Table II for the different stages); the numbers of oöcytes of each size group are plotted on the vertical axis. The vertical scale is plotted equally for all curves with exception of the one for Group I (10–36 μ). The values for Group I were so great that, in order to facilitate plotting all curves on the same graph, only the number of cells per half-section are plotted. All other curves are plotted on the basis of number of cells per whole section. The latter figure is hypothetical and was derived by multiplying by two the actual count of cells per half-section obtained during the investigation.

eggs available at time of fertilization (see Table II). The marked drop in the number of eggs of Group 148–180 μ (see Graph I) following fertilization (between Stages I and II) is a further indication of the large number of eggs fertilized. The fact that eggs over 184 μ remain

constant in number until after stages of segmentation probably indicates that growth of the next smaller group of eggs continues until after fertilization and thus maintains the same level, replacing those which had been fertilized. Normally, all eggs fertilized develop completely for degeneration of developing embryos is very scarce.

Atretic Follicles

With reference to the atretic follicles, only two generalizations are justified: (1) large degenerating follicles may occur throughout gestation but are more numerous in early and middle stages of gestation; (2) there is a noticeable increase in the number of small atretic follicles during the

TABLE III

Size of Oöcytes	Variation in Number of Oöcytes per Half Section	Extent of Fluctuation per Half Section
10– 36 μ	.2145–.8889	.6744
40– 72 μ	.0847–.2781	.1934
76–108 μ	.0259–.0909	.0650
112–144 μ	.0165–.0665	.0500
148–180 μ	.0085–.0635	.0550
184–216 μ	.0007–.0063	.0056

latter half of gestation, an observation that agrees with the general decrease of oöcytes of all sizes. On the whole, atresia of the eggs and their follicles does not undergo a cyclic behavior as evident as that found in the development of the oöcytes.

DISCUSSION

The only serious discussion of the variation of germ cells during gestation in viviparous teleosts is that of Turner (1937) on the ovoviviparous poeciliid fishes. In that article he compares a large number of poeciliids and makes several classifications on the basis of (1) number of broods of young in the ovary and (2) the relationship of the growing oöcytes to the stage of gestation. It is evident that *Neotoca,* a true viviparous fish, cannot be classified with any of the poeciliids reviewed by Turner in his interesting article.

In the *Gambusia affinis* type the growing oöcytes are held back until the birth of the current brood. Following that, they grow very rapidly from 0.5 mm. to as much as 1.5 mm. in order to attain maximal size before the next fertilization period.

In the *Lebistes reticulatus* type, the oöcytes are more or less grouped into different sizes, the largest eggs being nearly of maximal size at the end of gestation.

In the *Quintana atrizona* type, fertilization follows immediately upon birth of the previous brood, necessitating that eggs be fully grown and matured at time of birth of the preceding brood.

Two further types are those of *Poecilistes pleurospilus* and *Heterandria formosa* in which superfetation occurs, requiring that different sets of oöcytes grow, mature, and be fertilized while one or more broods of young are still growing within the ovary. Even in *Heterandria* where there are six levels of embryos, Turner recognizes one or two definite waves of eggs.

Neotoca, on the other hand, is unique in that there are no such waves or groups of embryos or oöcytes. The present analysis shows a condition different from any described by Turner for the poeciliids. Here the oöcytes show a continuous gradation in size throughout gestation. Unlike the *Gambusia* type, growth of oöcytes is not inhibited completely, merely somewhat retarded; throughout gestation there is a variable number of eggs of maximal size. Because of this, there is no necessity for a period of marked growth in the interval between birth of young and the following fertilization period. The different sizes of oöcytes merely continue their growth over the non-gestation period. It is true, however, that in *Neotoca* there is a general activation of oöcytes of all sizes in the resting interval because each size group shows a doubling or tripling in the number of cells before the following fertilization period.

Furthermore, since there are eggs of maximal size at the end of gestation in *Neotoca,* it cannot be compared to *Lebistes.* A second point of difference is that in *Lebistes* definite groups of oöcytes are recognized; in *Neotoca* the gradation seems to be complete.

Neotoca resembles *Quintana atrizona* more than the others since in both forms there are fully grown eggs at the end of gestation; however, *Neotoca* differs in that fertilization does not occur until seven days later whereas in the poeciliid, fertilization follows in a few hours. Furthermore, in *Quintana,* apparently the full complement of eggs is present at the end of gestation; in *Neotoca,* however, only between 20–25 per cent of the large eggs are present.

Finally, the condition of superfetation as in *Poecilistes* and *Heterandria* has been made possible in part by the maturing of eggs before the expulsion of the growing young from the ovary and in part by the removal of a physiological block that prevents such growth and fertilization. In view of this, superfetation technically could occur in *Neotoca* since there are eggs fully grown throughout gestation but, in addition,

there still is some physiological obstacle that normally prevents copulation and fertilization. Despite these normal conditions, Turner has reported (1940) occasional examples of superfetation in *Neotoca*. Furthermore, there was one ovary known to the writer in which several eggs had been fertilized within a few hours after the release of a previous brood but the eggs were retained most abnormally within the follicles and not evacuated as is normally true. This observation, in addition to Turner's finding of occasional cases of superfetation, shows that the phenomenon normally does not occur but may, in exceptional cases, get started. These exceptional cases in *Neotoca* apparently are always abortive.

If the germ cell cycle as it occurs in *Neotoca* is true of most or all Goodeidae, certainly an interesting difference occurs between two large and important families of viviparous teleosts.

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UPON THE PRESENCE AND DISTRIBUTION OF A CHROMATOPHOROTROPIC PRINCIPLE IN THE CENTRAL NERVOUS SYSTEM OF LIMULUS[1]

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Certain definite glandular bodies in arthropods have been shown to produce hormone substances. The more important of these are the crustacean sinus gland, located in the eyestalks of a majority of decapod crustaceans, and the corpora allata and the corpora cardiaca located in the vicinity of the esophagus in insects. These glands appear to be concerned with chromatic adaptations, growth, molt, reproduction, metamorphosis, and certain other functions.

Recently it has been pointed out that certain portions of the nervous system act in an endocrine capacity. This has been demonstrated by the work of Kopeć (1922), Brown (1933), Fraenkel (1935), Hosoi (1934), Brown and Ederstrom (1940), Wigglesworth (1940). Furthermore, histological studies of the nervous system of invertebrates as well as vertebrates have shown certain cells and cell clusters whose cytoplasm is definitely filled with granules or colloid, very strongly suggesting glandular activity (See Scharrer and Scharrer, 1940). This latter paper also describes the presence of such neurosecretory cells in *Limulus*.

With these facts in mind, we attempted to discover and measure an endocrine activity of certain tissues in the arachnid *Limulus*. The only work which had been done previously was that of Snyder-Cooper (1938). She was unable to discover any endocrine activity of the eyes, optic nerves, or central nervous system of *Limulus*, using the chromatophore system of *Palaemonetes vulgaris* as a test object. Since there are many chromatophore types in the crustaceans and recent work has demonstrated that the chromatophores show fundamental differences in their responses to known endocrine materials, it appeared worthwhile to reinvestigate the problem using *Limulus* with a number of chromatophore types other than those of *Palaemonetes* as an index of the presence of an active chromatophoric substance.

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The experiments reported here are restricted to a consideration of the activity of the central nervous system, because it appeared to be the most likely place of origin of an endocrine substance should any occur within the group, especially so since a portion of the nervous system has been shown to be active in both the other two classes of arthropods investigated. In this report a chromatophorotropic activity of the nervous system of *Limulus* will be described and it will be demonstrated conclusively that the active principle found within the nervous system is not uniformly distributed throughout the nervous tissue but shows a definite differential distribution. It may be seen in the paper following upon this one (Scharrer, 1941) that this differential distribution can be correlated with the distribution of neurosecretory cells within the central nervous system of the same species.

Materials and Methods

The experiments were commenced at the Marine Biological Laboratory at Woods Hole, Massachusetts, where freshly-caught *Limulus* and *Uca* were available, and completed at Evanston, Illinois, using *Limulus* and *Uca* which had been shipped from Woods Hole.

For the preparation of extracts of the nervous system, the live *Limulus* was quickly opened up, the nervous system removed and placed in sea water in a shallow container. The lateral nerves were trimmed away, leaving only their short stubs attached to the large nerve ring and the longitudinal chain of abdominal ganglia. In order to determine the effectiveness of various regions of the central nervous system, the system was cut with a scalpel into seven portions: section 1 included the anterior portion of the nerve ring; section 2, the lateral portions; section 3, the posterior portion of the nerve ring; sections 4, 5, 6 and 7 included respectively the first, second, third, and terminal ganglionic masses of the longitudinal cord. The relative positions of these cuts through the nervous system can be seen in Fig. 1. Each of the seven portions of the central nervous system was placed in a separate mortar and permitted to dry briefly; the nerve masses were then triturated thoroughly with pestles, in 2 cc. of sea water. It is appreciated that the total volume of extract obtained for the various nerve sections was somewhat different, due to the different sizes of the nerve masses. However, since the largest portion of the nervous system used in our experiments weighed less than .04 gram (except in one animal), this error was not considered an appreciable one. The extracts were then brought to a boil in order to precipitate out protein materials from the solution. The clear supernatant fluid was then used for assay purposes.

With each experimental series a control solution was prepared, consisting of a piece of muscle or digestive tract wall or gonad of approximately the same size as the largest nerve portion, extracted and treated in the same manner as the experimental solutions.

A sample of each extract, including the control, was injected into three blinded *Uca pugnax,* each *Uca* receiving an injection of approximately .05 cc. The injection was made into the basal segment of the third or fourth thoracic appendage.

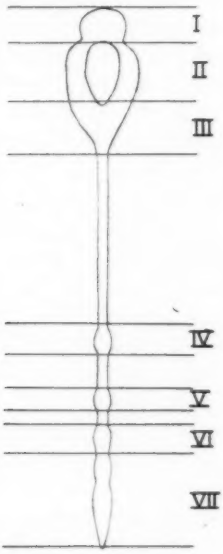

FIG. 1. Diagram of *Limulus* central nervous organs showing the sections of the system which were separately assayed for the chromatophorotropic principle.

Five experimental series were run. The chromatophore index for both black and white chromatophores was recorded at the beginning of each experiment and readings were taken at 15, 30, 45, 60 and 90 minutes. In the first experiment a large *Limulus,* approximately 30 cm. in length from the anterior end of the cephalothorax to the base of the telson, served as the source of nervous tissue. In the remaining four experiments smaller specimens of *Limulus* (about 12 cm. from anterior tip to base of telson) provided the nervous tissue.

Results

The results of these five experiments are shown in tabular form in Tables Ia and Ib. These tables give only the average chromatophore index for the three animals injected with each of the extracts, with the indices for the black and white chromatophores of course averaged separately. In these tables is shown also what has been called the coefficient of effectiveness of the various extracts. This coefficient of effectiveness we realize has only relative significance. It was calculated in the following manner: the sum of the averaged chromatophore indices for each of the two pigments at 15, 30, 45, 60 and 90-minute intervals was obtained. Since an extract having no effect upon the black chromatophores would leave these chromatophores with a chromatophore index of 1 (complete contraction) at each interval—hence a sum of 5—it was considered reasonable to subtract the constant 5 from the sum obtained following injections of active extracts. Similarly, since an extract which would leave the white chromatophores in an initially full dispersed condition (5) would yield a sum of 25, a true index of the effect of an active extract upon the white chromatophores would be the difference between the sum of the average indices and 25.[2] In brief, the coefficient of effectiveness for the black pigment is taken to be $x - 5$ and the coefficient of effectiveness of an extract in concentrating the white pigment is taken to be $25 - x$. In both of these instances x is equal to the sum of the averaged indices. This we believe to be a fair indication of the effectiveness of the extract since it takes into consideration both the magnitude and rate of the response, and, in many cases, duration of the response as well.

Another step was taken to make all the data of the five experiments comparable by obviating the differences which might exist as a result of the different sizes of *Limulus* used for the experiments. This was done by stating the effectiveness of the various portions of the nervous system in terms of the percentage of the effectiveness of Part III (the posterior portion of the ring), which was found in the earliest experiments to be obviously far more effective than any other portion of the nervous system. Thus, in Table I in the column "relative effectiveness" Part III has been arbitrarily assigned an activity of 100 and percentages have been calculated, on the basis of their coefficients of effectiveness, representing the effectiveness of the remaining portions of the nervous system in proportion to Part III.

[2] One difficulty arose which appeared to have no simple solution, namely, that the white chromatophores sometimes initially had their white pigment partially concentrated. It may readily be understood that to the extent to which this is true, the demonstrated differences in concentration of the substance in the nervous system assayed will be minimized.

The results of these experiments are summarized in Table II, in which the relative effectiveness of all the parts of the nervous system and of the control solution have been assembled and averaged. Inspection of these data indicates clearly that Part III is the most active, then,

TABLE Ia

Effect of Extracts upon Uca White

Exp.		0	15	30	45	60	90	Sum	Coefficient of Effectiveness (25−x)	Relative Effect
I	I	5.0	2.3	1.5	1.2	1.3	1.5	7.7	17.2	100.0
	II	5.0	2.5	2.5	1.75	1.25	1.0	9.0	16.0	93.0
	III	5.0	2.8	1.5	1.5	1.0	1.0	7.7	17.2	100.0*
	IV	5.0	2.0	3.0	1.8	2.0	2.0	10.7	14.2	82.5
	V	5.0	1.8	2.5	2.5	2.5	2.5	11.7	13.2	76.6
	C	5.0	5.0	5.0	5.0	5.0	5.0	25.0	0.0	
II	I	5.0	1.5	1.4	1.4	1.3	2.2	7.7	17.3	102.2
	II	4.8	2.2	1.9	1.5	1.2	1.1	7.9	17.1	101.2
	III	5.0	2.0	1.7	1.4	1.2	1.8	8.1	16.9	100.0*
	IV	5.0	3.2	3.1	2.8	2.7	3.5	15.3	9.7	57.4
	V	4.5	2.5	2.2	2.0	1.8	2.2	10.7	14.3	84.5
	C	4.0	4.2	4.2	4.1	4.0	4.0	20.5	4.5	26.6
III	I	4.7	2.7	2.7	2.7	2.7	3.0	13.8	11.2	66.2
	II	4.7	2.0	1.8	1.5	1.3	1.7	8.3	16.7	98.7
	III	5.0	2.3	1.8	1.6	1.3	1.1	8.1	16.9	100.0*
	IV	5.0	2.8	2.3	2.5	2.7	2.8	13.1	11.9	70.4
	V	5.0	3.0	3.0	3.4	3.8	4.5	17.7	7.3	43.2
	VI	4.7	3.3	3.3	3.1	3.0	3.4	16.1	8.9	52.6
	VII	3.4	2.0	1.8	1.8	1.8	2.3	9.7	15.3	90.5
	C	4.5	4.5	5.0	4.7	4.5	4.5	23.2	1.8	10.7
IV	I	3.7	1.8	1.8	1.7	1.5	1.5	8.3	16.7	91.3
	II	2.8	1.5	1.5	1.3	1.1	1.1	6.5	18.5	101.0
	III	2.3	1.7	1.5	1.3	1.1	1.1	6.7	18.3	100.0*
	IV	3.5	2.8	2.8	2.7	2.5	2.8	13.6	11.4	62.0
	V	3.8	2.8	2.7	2.7	2.7	3.1	14.0	11.0	60.1
	VI	3.0	2.8	2.7	2.7	2.8	2.8	13.8	11.2	61.1
	VII	3.2	2.7	2.7	2.8	3.0	3.0	14.2	10.8	59.0
	C	2.8	3.7	3.7	3.7	3.7	3.7	18.5	6.5	35.5
V	I	2.8	1.5	1.3	1.4	1.5	1.3	7.0	18.0	102.2
	II	3.2	2.0	1.7	1.4	1.3	1.5	7.9	17.1	97.2
	III	3.2	1.7	1.5	1.4	1.3	1.5	7.4	17.6	100.0*
	IV	2.7	2.7	2.5	2.4	2.3	2.6	12.5	12.5	71.0
	V	3.3	3.2	3.2	3.1	3.0	3.0	15.5	9.5	54.0
	VI	4.3	3.5	3.0	3.0	3.0	3.2	15.7	9.3	52.9
	VII	3.0	2.3	2.2	2.4	2.7	2.7	12.3	12.7	72.3
	C	3.3	3.2	3.3	3.7	4.0	3.5	17.7	7.3	41.5

TABLE Ib

Effect of Extracts upon Uca Black

Exp.		0	15	30	45	60	90	Sum	Coefficient of Effectiveness ($x-5$)	Relative Effect
I	I	1.0	2.5	2.5	3.3	2.7	3.0	14.0	9.0	81.8
	II	1.0	2.0	1.8	2.2	2.7	3.3	12.0	7.0	63.6
	III	1.0	2.3	2.5	2.7	4.3	4.2	16.0	11.0	100.0*
	IV	1.0	2.0	2.5	2.7	2.5	2.8	12.7	7.7	70.0
	V	1.0	3.0	2.5	3.0	3.2	2.3	14.0	9.0	81.8
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
II	I	1.0	1.7	1.8	1.8	1.8	1.7	8.8	3.8	38.8
	II	1.0	1.5	2.6	3.5	4.5	3.0	15.1	10.1	103.0
	III	1.0	1.8	2.5	3.7	4.4	2.4	14.8	9.8	100.0*
	IV	1.0	1.8	1.6	1.3	1.1	1.0	6.8	1.8	18.3
	V	1.0	1.3	1.3	1.3	1.3	1.0	6.2	1.2	12.2
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
III	I	1.0	2.2	2.3	2.3	2.2	2.7	11.7	6.7	58.8
	II	1.0	1.5	2.5	3.0	3.3	2.7	13.0	8.0	70.2
	III	1.0	1.5	3.2	3.7	4.2	3.8	16.4	11.4	100.0*
	IV	1.0	1.5	1.7	1.4	1.2	1.0	6.8	1.8	15.8
	V	1.0	1.5	1.3	1.1	1.0	1.0	5.9	0.9	7.9
	VI	1.0	1.3	1.2	1.0	1.0	1.0	5.5	0.5	4.4
	VII	1.0	1.5	1.5	1.3	1.0	1.0	6.3	1.3	11.4
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
IV	I	1.3	2.3	3.5	3.2	3.0	3.2	15.2	10.2	104.1
	II	1.0	1.3	1.8	2.7	3.7	4.3	13.8	8.8	88.6
	III	1.0	1.8	2.3	3.2	4.2	3.3	14.8	9.8	100.0*
	IV	1.0	1.7	1.7	1.7	1.7	2.0	8.8	3.8	38.8
	V	1.0	1.8	1.8	1.7	1.7	1.1	8.1	3.1	31.6
	VI	1.0	1.1	1.7	1.4	1.1	1.0	6.3	1.3	13.3
	VII	1.0	1.5	1.3	1.2	1.0	1.0	6.0	1.0	10.2
	C	1.0	1.2	1.0	1.0	1.0	1.0	5.2	0.2	2.0
V	I	1.0	2.0	2.8	2.8	2.8	3.2	13.6	8.6	90.5
	II	1.0	2.2	2.8	3.2	3.8	2.6	14.6	9.6	101.0
	III	1.0	1.8	3.0	3.2	3.5	3.0	14.5	9.5	100.0*
	IV	1.0	1.3	1.5	1.3	1.1	1.0	6.2	1.2	12.6
	V	1.0	1.0	1.0	1.0	1.2	1.0	5.2	0.2	2.1
	VI	1.0	1.2	1.2	1.2	1.0	1.0	5.6	0.6	6.3
	VII	1.0	1.5	1.5	1.5	1.5	1.3	7.3	2.3	24.2
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0

in order, Part II and Part I, and finally the nerve tissue of the ganglia of the longitudinal cord. With these data only, it is obviously impossible to determine the concentration of active principle within the various parts of the nervous system, since the portions varied considerably in size.

TABLE II

Relative Effects of Parts of Central Nervous System

Exp.	On White							
	I	II	III	IV	V	VI	VII	Control
I	100.0	93.0	100.0	82.5	76.6	—	—	0.0
II	102.2	101.2	100.0	57.4	84.5	—	—	26.6
III	66.2	98.7	100.0	70.4	43.2	52.6	90.5	10.7
IV	91.3	101.0	100.0	62.0	60.1	61.1	59.0	35.5
V	102.2	97.2	100.0	71.0	54.0	52.9	72.3	41.5
Av.	92.4	98.2	100.0	68.7	63.7	55.5	73.9	22.8
Exp.	On Black							
	I	II	III	IV	V	VI	VII	Control
I	81.8	63.6	100.0	70.0	81.8	—	—	0.0
II	38.8	103.0	100.0	18.3	12.2	—	—	0.0
III	58.8	70.2	100.0	15.8	7.9	4.4	11.4	0.0
IV	104.1	83.6	100.0	38.8	31.6	13.3	10.2	1.0
V	90.5	101.0	100.0	12.6	2.1	6.3	24.2	0.0
Av.	74.8	84.3	100.0	31.1	27.1	8.0	15.3	0.2

To make this calculation, it was necessary to know two more facts: first, the volume of the various parts of the nervous system extracted, and second, the relation between the concentration of active principle within an extract and the calculated coefficients of effectiveness.

In order to answer the first problem, the various parts of the nervous system used were individually weighed prior to their extraction. The results of these weighings are found in Table III.

TABLE III

Weights of Parts

No.	Part	Exp. IV Wgt. Gms.	Exp. V Wgt. Gms.	Average
I	Anterior nerve ring	.0291	.0350	.032
II	Lateral nerve ring	.0310	.0347	.033
III	Posterior nerve ring	.0219	.0306	.026
IV	First ganglion	.0047	.0044	.0046
V	Second ganglion	.0041	.0030	.0036
VI	Third ganglion	.0032	.0061	.0047
VII	Fourth ganglion	.0021*	.0073	.0047
Control	Muscle	.0186	.0246	.022

* Part of ganglion was lost in the preparation of the ganglion for weighing, therefore average should be higher.

An experiment was then designed to determine the relationship between the coefficients of effectiveness and the concentration of the active principle in the extracts. In two of the preceding five experiments, a portion of the extract prepared from Part III was set aside in order to determine the effects of known dilutions upon the two chromatophoric types of *Uca*. In this experiment the extract of Part III was diluted to half its original concentration, then one-fourth, one-eighth, one-sixteenth, one-thirty-second, and one-sixty-fourth. Each dilution stage was injected into three *Uca* just as in the original assay experiments and the coefficient of effectiveness of each dilution was calculated in the same way. The results were expressed as percentages, keeping the original concentration of Part III of the nervous system as 100 per cent with the various dilution stages decreasing according to their coefficients. The results of this experiment with respect to the white chromatophores are seen in Table IVa, and for the black chromatophores in Table IVb.

These data were used for calculating the relative concentration of active principle throughout the nervous system of *Limulus*, as follows: a graph was constructed, the abscissa of which represented the logarithm of the relative concentration and the ordinate the effectiveness in terms of percentage of the original concentration. The results are plotted in Fig. 2. The best smooth curves possible have been drawn through the two series of eight points. With the aid of these plots, it was possible to determine the relative concentration of active chromatophorotropic principle by locating the percentage response on the graph and reading the log of the relative concentration on the abscissa. Using this technique, the relative concentration of active principle for the various portions of the nervous system used in this experiment were calculated.

In Fig. 3 we have plotted together, upon the same abscissa (the segments of the nervous system) but on different ordinates, the weights of the various experimental sections of the nervous system and the apparent relative total quantity of active principle in each part of the nervous system. We have assumed that the specific gravity of all portions of the nervous system is roughly constant, which seems reasonable.

Now the apparent relative quantity of active principle in each part of the nervous system was divided by the weight in grams of that particular portion, and a figure was obtained which indicates the relative concentration of the active principle in these portions. These calculations are summarized in Table V. Inspection of this table indicates that Part III of the nervous system has double the concentration of Part II and nearly four times the concentration of Parts I, IV, V and VII, and nearly ten times the concentration of Part VI.

TABLE IVa

Effect of Dilution on White

	15	30	45	60	90	Sum	Coeff.	Percentage Relative Effect	
Exp. IV									
1	1.7	1.5	1.3	1.1	1.1	6.7	18.3	100.0*	
1/2	2.0	1.7	1.5	1.3	1.5	8.0	17.0	93.0	
1/4	2.0	1.5	1.6	1.7	2.2	9.0	16.0	87.5	
1/8	1.7	1.8	1.8	1.8	1.7	8.8	16.2	88.5	
1/16	2.2	2.5	2.6	2.7	2.3	12.3	12.7	69.4	
1/32	2.3	2.7	2.5	2.3	2.5	12.3	12.7	69.4	
1/64	3.7	3.3	3.7	4.0	4.0	18.7	6.3	34.4	
0	3.7	3.7	3.7	3.7	3.7	18.5	6.5	35.5	
Exp. V									Average
1	1.7	1.5	1.4	1.3	1.5	7.4	17.6	100.0*	100.0
1/2	1.8	1.5	1.4	1.3	1.3	7.3	17.7	100.8	96.9
1/4	2.2	2.3	2.0	1.7	1.5	9.7	15.3	87.0	87.3
1/8	2.2	2.0	1.9	1.8	2.2	10.1	14.9	84.7	86.6
1/16	2.2	2.2	2.0	1.8	1.8	10.0	15.0	85.3	77.4
1/32	3.5	3.2	3.2	3.2	3.2	16.3	8.7	49.5	59.5
1/64	3.0	2.5	2.6	2.6	2.6	13.3	11.7	66.5	50.5
0	3.2	3.3	3.7	4.0	3.5	17.7	7.3	41.5	38.5

TABLE IVb

Effect of Dilution on Black

	15	30	45	60	90	Sum	Coeff.	Percentage Relative Effect	
Exp. IV									
1	1.8	2.3	3.2	4.2	3.3	14.8	9.8	100.0*	
1/2	2.2	2.3	2.7	3.2	3.2	13.6	8.6	87.7	
1/4	2.8	3.8	3.5	3.2	3.0	16.3	11.3	113.0	
1/8	2.3	2.3	2.4	2.5	2.3	11.8	6.8	69.4	
1/16	1.8	1.7	1.6	1.5	1.3	7.9	2.9	29.6	
1/32	1.5	1.3	1.3	1.3	1.2	6.7	1.7	17.3	
1/64	1.2	1.3	1.3	1.3	1.2	6.3	1.3	13.3	
0	1.0	1.2	1.0	1.0	1.0	5.2	0.2	2.0	
Exp. V									Average
1	1.8	3.0	3.3	3.5	3.0	14.3	9.3	100.0*	100.0
1/2	2.0	2.3	2.3	2.3	2.6	11.5	6.5	69.9	78.8
1/4	1.5	1.5	1.6	1.7	1.7	8.0	3.0	32.2	72.6
1/8	1.7	1.7	1.9	2.2	1.7	9.2	4.2	45.1	57.2
1/16	1.5	1.5	1.6	1.8	1.7	8.1	3.1	33.3	31.5
1/32	1.3	1.3	1.5	1.7	1.5	7.3	2.3	24.7	21.0
1/64	1.0	1.0	1.0	1.3	1.0	5.3	0.3	3.2	8.2
0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	1.0

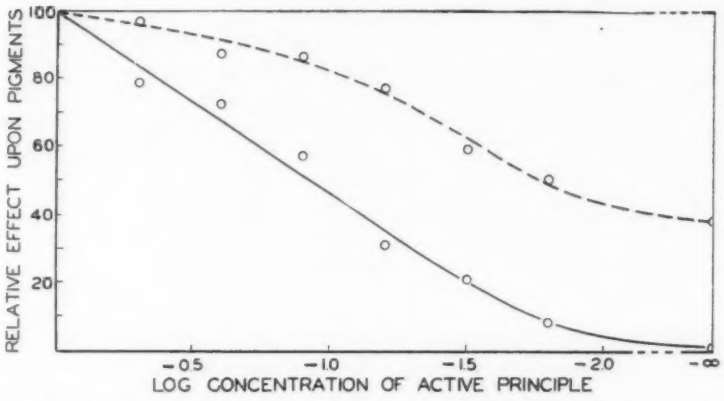

FIG. 2. The relationship between the log concentration of the active principle and the relative effectiveness upon the *Uca* white (dashed line) and black (solid line) pigments.

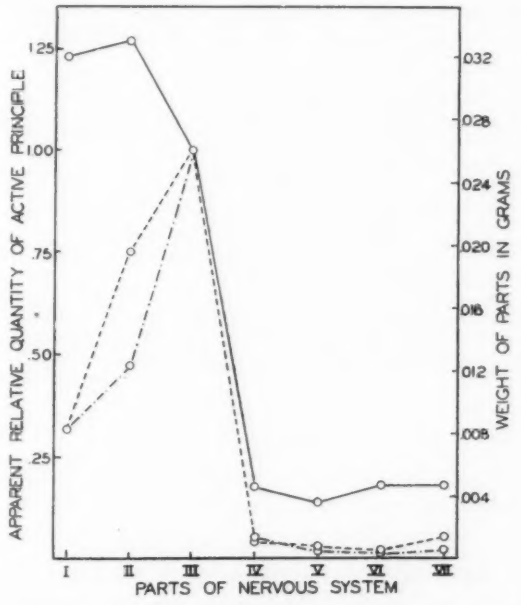

FIG. 3. Plotted together for comparison are the apparent relative quantity of white pigment concentrating principle (dashed line), of black pigment dispersing principle (dot-dash line), and weights of each of the seven assayed parts of the *Limulus* nervous system.

TABLE V

Part	Apparent Rel. Quant. Black Disp. Principle	Apparent Rel. Quant. White Conc. Principle	Weight of Part (gms.)	Rel. Conc. Black Disp. Principle	Rel. Conc. White Conc. Principle	Average
I	0.32	0.32	0.032	10.0	10.0	10.0
II	0.47	0.75	0.033	14.3	22.8	18.6
III	1.00	1.00	0.026	38.5	38.5	38.5
IV	0.053	0.043	0.0045	11.8	9.6	10.7
V	0.045	0.034	0.0035	12.9	9.7	11.3
VI	0.016	0.022	0.0047	3.4	4.7	4.1
VII	0.024	0.054	0.0047	5.1	11.5	8.3

Effects of the Chromatophorotropic Principle of Limulus Nervous System upon Certain Other Chromatophore Types

In the light of the work of Snyder-Cooper (1938) in which she found no apparent chromatophorotropic effects of injection of *Limulus* nervous system extracts upon *Palaemonetes* chromatophores, we believed it worthwhile to repeat her experiments. We were also unable to show any response of either the red or the white chromatophores of *Palaemonetes* to these extracts.

Extracts of the nervous system of *Limulus* were tested upon isolated chromatophores of *Cambarus*, according to the technique of Brown and

TABLE VI

	Uca		*Cambarus*		*Palaemonetes*	
	Black	White	Red	White	Red	White
Sinusgland *Cambarus*	—	—	C	D	—	—
Sinusgland *Uca*	D	D	—	—	C	D
Nervous system *Cambarus*	—	—	C	C	—	—
Corpus cardiacum insects	D	C	C	O	—	—
Brain insect	—	—	O	C	—	—
Nervous system *Limulus*	D	C	O	C	O	O

Meglitsch (1940) and it was found that the chromatophorotropic principle of *Limulus* was very effective in concentrating white pigment, but was entirely without effect upon the red. Furthermore, as Fig. 4 indicates, the relative effectiveness of Parts I through VII was approximately the same upon *Cambarus* white chromatophores as upon *Uca* black and white.

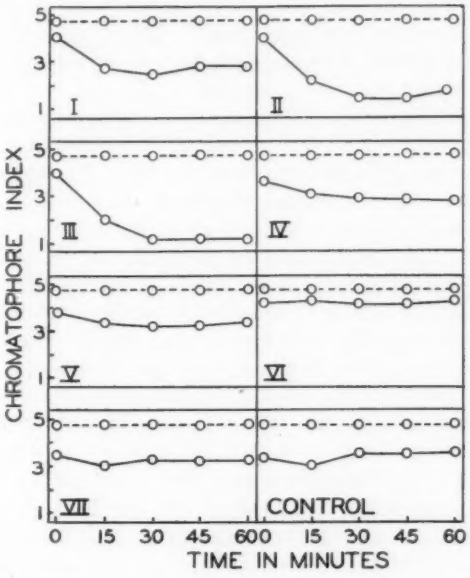

FIG. 4. Relative effects of the parts of *Limulus* nervous system upon crayfish (*Cambarus immunis*) white chromatophores (solid line) and red ones (dashed line). Five upon the ordinate indicates a fully dispersed pigment mass, and one, a fully concentrated pigment mass.

DISCUSSION

Comparison of the Chromatophorotropic Material of Limulus with Chromatophorotropic Materials of Other Arthropods

Table VI has been prepared to show the effects of various arthropod organ extracts upon six types of crustacean chromatophores. This table is admittedly incomplete and although it would be both interesting and instructive to have the gaps filled, it is still possible to draw certain conclusions from it as it is.

In comparing the action of the *Limulus* nerve cord extract with that of the sinus gland extracts of *Uca* and of *Cambarus,* we see that upon *Palaemonetes* red and white chromatophores the *Limulus* extract has no effect, whereas definite and characteristic effects are produced by sinus gland. Upon *Cambarus* red, sinus gland exercises a strong concentrating influence; this is apparently entirely lacking in the *Limulus* extract. Upon *Cambarus* white chromatophores, on the other hand, both the extracts are effective but result in opposite responses of the chromatophore. Similarly, the two extracts have opposite actions upon *Uca* white chromatophores,[3] but upon *Uca* black the activity of the two substances is qualitatively the same.

It seems to us reasonable to assume that this is a similar response of the chromatophore to two chemically different materials, in other words, a non-specific chromatophore reaction. We assume this inasmuch as our experiments suggest that both the white-concentrating action and the black-dispersing action were produced by the same material.

At first it may seem rather extraordinary that *Limulus* should be suspected of having a chromatophorotropic material because of the absence of functional chromatophores in this group of animals, but many other organisms without physiological color change (cockroaches, etc.) possess active corpora cardiaca and sinus glands, and there is abundant evidence accumulated that the chromatophorotropic action of these organs is only one of a number of functions, many of which are far more basic in the life processes of the animals than that of chromatic adaptation. We have utilized the chromatophore response as a test method with a full appreciation of this fact.

There are some who will contend that the materials with which we dealt are nothing more than materials resulting from the mechanical destruction of nerve tissue and possess no normal endocrine function within the organism. This seems highly unlikely considering a number of observations such as the restriction of a specific material to the commissural ganglion of *Crago* and the restriction of a specific action to the corpora cardiaca of insects, and finally, in this research, to a definite demonstration that the material is not uniformly distributed throughout the nervous system, some portions of the nervous system showing roughly ten times the concentration of active principle shown by others. There is some evidence, however, that the material in question in *Limulus* is not produced by a single locus within the nervous system and then

[3] Abramowitz (1937) states that *Uca* eyestalk extract concentrates white pigment in *Uca pugnax*. We have been unable to confirm this observation of Abramowitz and, on the contrary, find that *Uca* eyestalk extract has a definite and striking dispersing action upon white pigment, just as seen in *Palaemonetes* and *Cambarus*.

distributed out from this center by diffusion because it was demonstrated in a brief experiment that the longitudinal commissure connecting the posterior end of the nerve ring with the first ganglion of the longitudinal chain showed significantly lower concentration of active principle (practically no effect) than either the posterior portion of the nerve ring or the ganglion at its opposite end. In the case of diffusion, a smoothly gradual decline in activity would be expected. The increased activity at the posterior tip of the central nervous system also argues against the diffusion of the material out from a single center. Therefore we are inclined to believe that this differential distribution of activity in the nervous system is the result of a differential distribution of cells actively engaged in the production of the substance.

The Probable Number of Hormonal Substances in Limulus Nervous System

If one examines Fig. 3 one is impressed with the parallel nature of quantitative distribution of the black and white pigment-concentrating principles. The differences which occur are not only readily within the experimental error but also are even astonishingly close to one another. On the basis of these data there is no justification for any assumption other than that these two pigments are being affected by one and the same substance. An examination of Fig. 4, showing the effects of the various parts of the nervous system of *Limulus* upon the white chromatophores of *Cambarus*, shows a quantitative gradation of activity of the various parts quite parallel to those shown in Fig. 3 for the *Uca* chromatophores. Again there is apparently no reason for assuming anything other than that the substance active upon *Cambarus* white chromatophores is the same substance responsible for influence on *Uca* chromatophores. We realize the danger of drawing any conclusions upon negative evidence and hence conclude only that there is no suggestion for more than one chromatophorotropic principle in *Limulus* nervous system. However, just as Snyder-Cooper failed to show the presence of any chromatophorotropic principle in *Limulus* using *Palaemonetes* red and white chromatophores, so is it quite possible that utilizing other chromatophores than we have tried will demonstrate the presence of other hormones than we have been able to demonstate.

The action of *Limulus* extract is qualitatively unlike that of extracts of the commissural ganglia and other nervous organs in *Cambarus*, as shown by their effect upon *Cambarus* red chromatophores. The *Cambarus* extracts show an extremely potent activity in concentrating the red pigment while *Limulus* extracts show no effect. On the other hand, the

effects of these two extracts are identical upon *Cambarus* white chromatophores. Two alternative explanations are possible: (1) that the white-concentrating principle from these two sources is similar and that *Cambarus* nervous system possesses in addition a red pigment-concentrating principle, or (2) one could assume that each extract possesses a single principle which is structurally different in the two cases. Again, *Limulus* nerve organ extract differs from extracts of the corpora cardiaca of insects in having a different action upon both red and white chromatophores of *Cambarus*. Finally, in comparing the activity of insect brain and the activity of *Limulus* nervous system, one finds a qualitatively similar action of these two extracts upon both red and white chromatophores of *Cambarus*. Neither possesses an effect on the *Cambarus* red pigment and both exercise a white pigment-concentrating action. Of course it is too soon even to suspect that these latter two substances are identical and further conclusions cannot be drawn until more properties of these two substances have been shown to be identical.

A consideration of these results brings us to a complete realization that a unitary theory of hormonal control of chromatophores in crustaceans—and even more in arthropods in general—is completely untenable. There are undoubtedly several different chromatophorotropic materials found within the various groups, but it is not beyond the realm of possibility that certain threads of similarity or continuity can be woven through various active tissues and their secreted principles in this phylum of animals.

It can be calculated readily that the posterior portion of circumesophageal nerve ring of *Limulus* is still effective when diluted in nearly 5000 times its volume of salt solution. The active secreting cells undoubtedly occupy a very small percentage of the volume of the nervous tissue and consequently, in terms of the ratio of neuro-glandular tissue to volume of extract, the maximal dilution value would be in the hundreds of thousands or even millions.

Summary

1. A principle influencing pigment concentration in *Uca* chromatophores is found in extracts of the central nervous system of *Limulus polyphemus*. This principle is not uniformly distributed through the central nervous system of *Limulus* but is concentrated in the ganglionic masses, with the greatest quantity in the posterior portion of the circumesophageal nerve ring. The lateral portions of the nerve ring show approximately one-half the concentration of the posterior portion and

all the remaining portions of the nervous system show from one-quarter to one-tenth the concentrations of the posterior portion of the nerve ring.

2. The distribution of the principle influencing *Uca* white pigment appears to be identical with that producing dispersion of the *Uca* black pigment and concentration of *Cambarus* white pigment. Hence it is concluded that all three of these effects are brought about by one and the same principle.

3. Certain physiological properties of the chromatophorotropic material from the nervous system of *Limulus* were compared with corresponding properties of certain other invertebrate hormones and it was found that the *Limulus* chromatophorotropic principle is physiologically unlike any other known arthropod hormone substance with the possible exception of insect brain extract.

4. It is calculated that an extract of the posterior portion of the circumesophageal nerve ring is still effective when diluted in nearly 5000 times its volume of salt solution.

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NEUROSECRETION

IV. Localization of Neurosecretory Cells in the Central Nervous System of Limulus

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From an investigation, still under way, of the neuroglandular cells in the central nervous system of the horseshoe crab (*Limulus*), mention of which has been made in a previous review article (Scharrer and Scharrer, 1940), some data concerning the occurrence, localization, and numerical distribution of these cells are reported here. The publication of these data appears to be timely in view of the findings of Brown and Cunningham, reported in the preceding paper of this journal (1941). These authors demonstrate the presence of a chromatophorotropic principle within the nervous centers of *Limulus* and calculate the concentration of this substance in different, separately tested portions of the nervous system. They conclude that the chromatophorotropic principle is probably produced in definite groups of cells within the central nervous system. The agreement between the results of the physiological work of Brown and Cunningham and the morphological findings to be reported here suggests the possibility that in *Limulus* the neurosecretory cells are the source of the chromatophorotropic principle.

Material and Methods

Altogether forty adult male and female specimens of *Limulus polyphemus* were studied. Most of the material was preserved during the summer months of 1937, 1939, and 1940 at the Marine Biological Laboratory, Woods Hole.[1] Additional specimens were obtained alive from the New York Aquarium,[2] and were preserved for histological study at various intervals during the years 1938–1940. Furthermore, a few young specimens of *Limulus polyphemus* and two female *Limulus moluccanus*, one of them from Penang (Malay Peninsula) fixed in the summer

[1] For the use of research facilities during these periods the author is indebted to the Rockefeller Foundation and to the Rockefeller Institute for Medical Research, New York.

[2] I am obliged to Dr. C. W. Coates for his friendly assistance in obtaining this material.

of 1938, the other from the mangrove swamps at Chandipur (Orissa, India),[3] were included in this study.

In order to obtain comparable results, the same histological technique was used in all cases. The central nervous system, which in adult specimens is of considerable size, was carefully dissected out and was fixed in Zenker-formol. It was subsequently embedded in celloidin (nitrocellulose) and horizontal serial sections of 20 μ were stained with Foot's modification of Masson's trichrome method.

The various degrees of neurosecretory activity to be found in different parts of the central nervous system as well as in the different specimens of *Limulus* studied were estimated by counting in every section all cells containing secretory colloid. Thus undoubtedly a certain percentage of the cells was counted more than once because the vacuoles containing colloid are often large enough to appear in more than one section of 20 μ thickness. This is not to be considered an error of consequence, because this method of recording colloid whenever it appears in the sections, even if it belongs to the same cell, takes account of the volume of colloid as a whole rather than of the actual number of cells containing colloid inclusions. For purposes of comparing the secretory activity of different regions of the central nervous system this method appears to be satisfactory.

Observations

The Histological Appearance of the Neuroglandular Cells

It is not intended here to describe the cytological characteristics of cells considered as having a glandular function or to investigate the steps in which the transformation of a nerve cell into a gland cell takes place. In the present study the concern is only with such cells as are believed to represent the fully developed type of neurosecretory cell characteristic of *Limulus*. This cell type is fairly uniform and easily recognizable. The cells contain large masses of a colloidlike substance (Fig. 1) which appears homogeneous in sections treated in the manner described before. The substance stains green with the light green component of the Masson trichrome stain and seems to have physical properties not unlike those of the colloid of the thyroid gland (for corresponding parallels see also Hanström, 1941). This similarity is also suggested by vacuoles in the periphery of the colloid masses in the cells of *Limulus* which remind the observer of similar vacuoles seen in sections of thyroid colloid. Apparently this colloid mass pushes the nucleus and the cytoplasm aside and

[3] The efficient cooperation of Dr. Baini Prashad, Director of the Indian Museum, Calcutta, India, is gratefully acknowledged.

takes up a large space in the cell. These conspicuous colloid-carrying cells were counted.

Cells of this kind have also been found in the ganglia of cockroaches (Scharrer, 1941). However, in the cockroach they represent one of several kinds of neurosecretory cells, whereas in *Limulus* only this one type is encountered. There are in *Limulus* cells the appearance of which suggests that they represent phases preceding the fully developed "mature" neurosecretory cell but their description is not undertaken here, since their relation, if any, to the colloid-containing cells is still undetermined. From a histological point of view they certainly seem insignificant as a possible source of secretion material when compared with the

FIG. 1. Three neurosecretory cells, each in its capsule, from the circumesophageal ring of *Limulus polyphemus* with partly vacuolated colloid indicated in solid black. Zenker-formol, nitrocellulose, 20 μ, Masson.

cells containing the large masses of colloid. The contrast between the ordinary nerve cells, including those in which differences in stainability of the cytoplasm etc. suggest that they may be in a state of transformation into glandular elements, and the cells counted here as neurosecretory cells is always so definite that no doubt arises as to which cells should be included in the counts. *Limulus* is particularly favorable in this respect for the kind of investigation carried out here.

It should be repeated that the cells containing a homogeneous mass of colloid with varying numbers of marginal vacuoles are always observed when the histological technique described before is used. The fact that the appearance of the colloid is somewhat different after treatment by different methods is of no concern here where only the amount and distribution of the secretory elements are of interest.

The Localization of the Neuroglandular Cells within the Central Nervous System

The central nervous system of *Limulus* consists of the circumesophageal ring, situated in the cephalothorax, and the abdominal ganglia (Fig. 2). The ring contains the "brain" and a number of thoracic ganglia of the ventral cord, designated in Fig. 2 as Nos. 1–8, beginning with the cheliceral ganglia. There are, in addition, eight pairs of abdominal ganglia, only the first four or five of which are well defined, the remaining pairs being fused together (Patten and Redenbaugh, 1900).

With the exception of the corpora pedunculata which make up about three-fourths of the brain, all parts of the central nervous system of *Limulus* contain neurosecretory elements among the ordinary nerve cells. The distribution of the neurosecretory cells varies, however, with respect to the different ganglia and their total number shows the greatest variations from specimen to specimen.

A most "active" neurosecretory region is the posterior part of the circumesophageal ring, i.e. the area of the thoracic ganglia No. 6 and No. 7. Here the neuroglandular elements are found in clusters which may constitute a considerable proportion of the total mass of cells in the ganglion. These clusters of glandlike cells are arranged symmetrically with respect to the mid-sagittal plane (Fig. 3).

More anteriorly in the ring the neuroglandular cells become less and less frequent. They may even be absent in these portions, particularly in those specimens which, on the whole, show less neurosecretion than others. If present, neuroglandular cells in ganglia No. 1 to No. 5 appear single or in small groups of two or three. Their approximate number and position are the same on both the left and right side of the ring. Of all neurosecretory cells found in the circumesophageal ring only about one-tenth or less lie in the two anterior thirds, the majority being concentrated in the posterior third of the whole ring.

The abdominal ganglia, counted together, contain roughly on the average twice or three times as many neurosecretory elements as the corresponding ring. The numbers of neuroglandular cells counted in the circumesophageal ring and in the abdominal ganglia of four specimens are given here as examples:

Circumesophageal ring	117	206	264	516
Abdominal ganglia	340	442	861	1978

Thus as a rule and within limits, from the degree of neurosecretory activity in a given ring the activity in the abdominal ganglia of the same specimen can be predicted. The individual abdominal ganglia do not

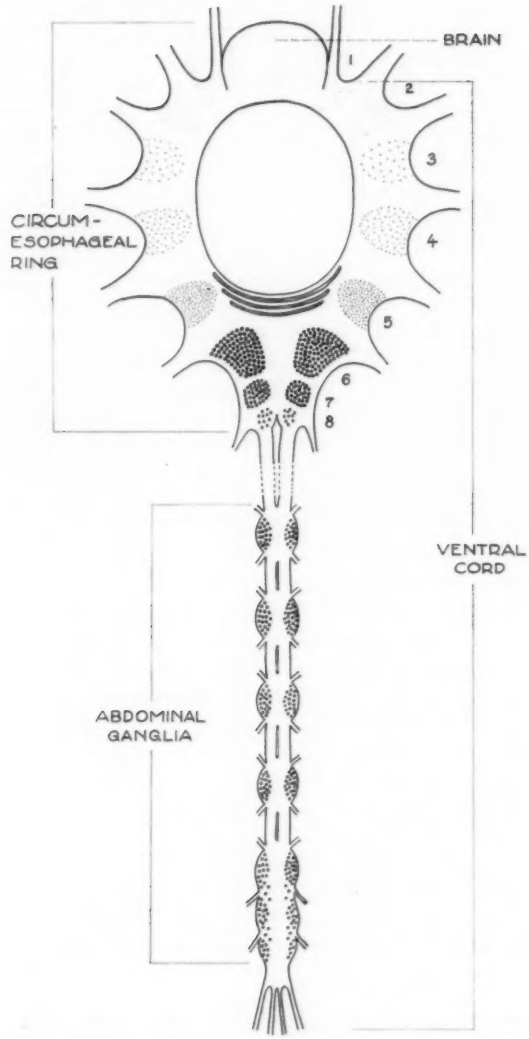

FIG. 2. Diagram of the central nervous system of *Limulus*. Numbers 1–8 are the ganglia of the circumesophageal ring. The areas where neurosecretory cells may be found are dotted. Coarser dots indicate a higher degree of neuroglandular activity than finer dots.

seem to differ significantly from each other in number of neurosecretory cells.

Individual Variations of Neurosecretory Activity

Whereas the central nervous system of a few young specimens of *Limulus polyphemus* (width of carapace from 5 to 8 cm.) does not con-

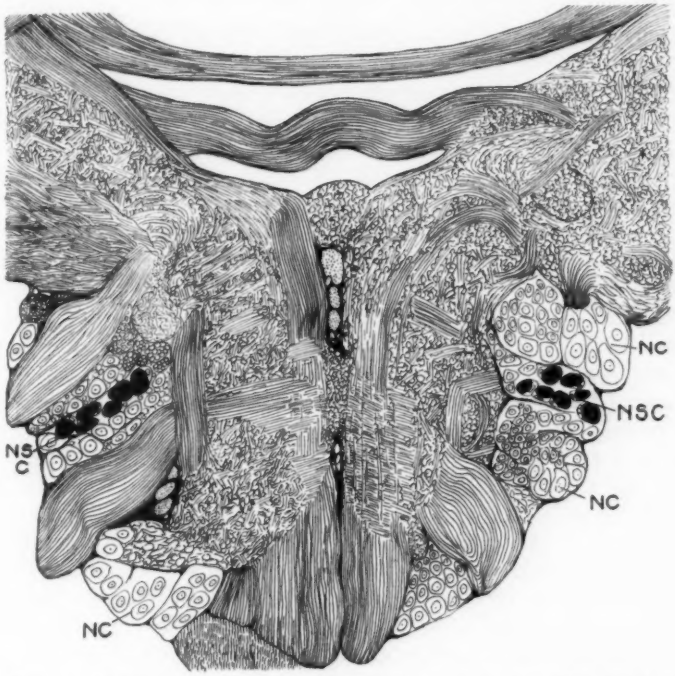

FIG. 3. Horizontal section through posterior part of the circumesophageal ring of *Limulus polyphemus*. *NSC*, neurosecretory cells; *NC*, nerve cells. The colloid of the neurosecretory cells which appears green with the histological technique used is indicated by solid black. Note symmetry of neurosecretory cell groups. Zenker-formol, nitrocellulose, 20 μ, Masson.

tain neuroglandular cells, these cells are present in all adults studied thus far. This concerns animals from different geographical sources as well as from two different species. Furthermore, neurosecretion is found in both male and female *Limulus*.

The degree of neurosecretory activity, however, varies considerably from specimen to specimen. In some, the entire central nervous system is found to contain only one or two neuroglandular cells, in others over

a thousand such cells may be counted by the method described. The highest count thus far made, 2494, was in a large female. Between these extremes are such counts as 457, 648, 1125. These figures are given only to show the wide variation in view of which the errors necessarily involved in the calculation method employed here appear of minor importance.

The question may be asked next whether there exist any relations between the degree of neurosecretory activity and certain known factors. The following observations were made:

(1) A 24-hour cycle of secretion does not seem to exist. The histological appearance of neuroglandular cells is the same in different specimens fixed at various hours of the day.

(2) Neurosecretion in *Limulus* is not restricted to one time of the year, such as, for instance, the breeding season. None of the summer specimens contains more neuroglandular cells than the animals with the highest counts fixed in January, March, or November.

(3) The degree of neurosecretory activity in males and females is not essentially different. On the average, however, smaller numbers of neuroglandular cells are found in males, but this may be explained by the smaller average size of the male central nervous system.

(4) So far the only factor of some importance appears to be the age of the animals, as expressed by the size, i.e. the width of the carapace. As a rule, the larger specimens show a more active state of neurosecretion. The *Limulus* with the highest count of neurosecretory elements (almost 2500) was the largest specimen studied, a female of 32 cm. width which contained many and large eggs. On the other hand, none of five females under 23 cm. width showed more than about five secreting cells. Also among the males the largest specimen examined yielded the highest count but that does not exclude the fact that one or another small male may be encountered in a comparatively active state of neurosecretion. Thus, for instance, in the smallest male among a dozen studied, having a carapace-width of 16.5 cm., the relatively high number of 648 secreting cells was found. For comparison it may be noted that in two other male specimens these counts were made: carapace 19 cm., 5 cells; carapace 20 cm., 205 cells.

Although the existing evidence is not entirely conclusive, the extent to which nerve cells are engaged in secretory activity seems to run grossly parallel with age as expressed in the size of the animal. This fact has to be taken into account in attempts to influence experimentally the ratio of neuroglandular cells. Such experiments as have been carried out up to the present time were unsuccessful. In one extensive series extracts were made from the circumesophageal ring of the central nervous

system of *Limulus* by grinding it with sand and sea water. The extracts from several, for instance five, different specimens of varying sizes and different sex, were pooled and injected into the body cavity of male and female specimens over varying periods of time. Approximate estimates were made of the total number of rings injected into each experimental animal. Thus, for instance, each of several animals got the equivalent of 17 rings over a period of one month. The counts made in such animals kept well within the limits of the normal variation:

♂	21 cm.	1073	♀	23 cm.	1
	21 "	617		23.5 "	22
	22 "	446		28 "	492

From the number of experiments done by the method described it can be safely concluded that sea water extracts from the neurosecretory cells of *Limulus* do not influence the number of cells engaged in secretion. It may be added also that no change in the histological appearance of these cells has been observed.

Similarly ineffectual were injections of pilocarpin. Of a 1 per cent solution in two specimens as much as 19 cc. were administered by means of two injections, both given on one day. Smaller amounts were injected in others. Again, the counts and the histological appearance of the cells gave no indication that pilocarpin acts on the neuroglandular cells in the concentrations used here which, in view of their general effect on the animals, may be considered as near the toxic ones.

Discussion

The data reported here on the occurrence and distribution of neurosecretory cells in the central nervous ganglia of *Limulus* correlate well with the findings of Brown and Cunningham (1941). The two authors describe a chromatophorotropic principle in extracts from all parts of the central nervous system of *Limulus polyphemus*. From the effect on crustacean chromatophores they conclude that the concentration of the active material varies with respect to different, separately tested parts of the central nervous system. The present histological study demonstrates the presence of nerve cells offering the picture of gland cells in all portions of the nervous system tested by Brown and Cunningham. The distribution of these elements in the nervous tissue varies in different regions, and this variation corresponds well with the distribution of the chromatophorotropic material found by these two authors. This correlation becomes particularly evident in the different portions of the circumesophageal ring. Its posterior third for which Brown and Cunningham report the greatest concentration of the active principle con-

tains the majority of neurosecretory elements present in the ring. In the lateral sectors where the relative chromatophorotropic activity shows a considerable decrease, many fewer cells are found to contain secretory colloid. The anterior portion of the ring with relatively the lowest action on crustacean chromatophores contains glandular cells only occasionally. The connectives between the circumesophageal ring and the abdominal ganglia did not yield the chromatophorotropic principle; neither colloid nor neuroglandular cells are found in these connectives.

Considering that the estimate of the concentration of the chromatophorotropic principle in the nervous system of *Limulus* must necessarily be approximate, and that the cell counts may mean little in terms of function, the correlation between physiological and anatomical findings demonstrated here seems all that can be expected.

Within its obvious limitations this correlation consequently suggests that the neurosecretory cells in the central nervous system of *Limulus* may be considered as the source of the chromatophorotropic principle. If this proves to be correct the functional significance of neurosecretion assumes a new aspect. Thus far only one function has, with good evidence, been attributed to neuroglandular cells, namely the production of a hormone controlling molting in insects (Wigglesworth, 1940).

Summary

The occurrence, localization, and quantitative distribution of neurosecretory cells in the central nervous system of *Limulus* have been described. These anatomical findings are in good agreement with the physiological data of Brown and Cunningham (1941), who demonstrate the presence and distribution of a chromatophorotropic principle in the nervous system of this animal. Therefore, the neurosecretory cells of *Limulus* may be considered as the source of a substance influencing color change in crustaceans.

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THE ACTION OF ACETYLCHOLINE, ATROPINE AND PHYSOSTIGMINE ON THE INTESTINE OF DAPHNIA MAGNA

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Introduction

The effects of acetylcholine when administered to animals under experimental conditions have recently afforded valuable information which suggests the possibility that acetylcholine is acting as a chemical transmitter of nervous impulses from nerve endings to certain organs of the body. In view of the excitatory action of acetylcholine which has been demonstrated on the heart of crustaceans (Welsh, 1939 *a* and *b*) and the influence of this substance on the autotomy of certain members of this group of animals (Welsh and Haskin, 1939), it appears worth while to investigate the action of this drug on the intestine of a cladoceran, a problem heretofore unexplored. Although there is nothing in Cladocera which corresponds morphologically to the autonomic nervous system in vertebrates, the intestine of these animals is subject to accelatory and inhibitory nervous influences. If the intestine of *Daphnia magna*, for example, is touched with a very fine glass needle at the bend of the digestive tube where the intestine enters the stomach, the heart immediately stops beating and the posterior region of the intestine commences to exhibit powerful intestinal contractions. After a certain period, depending on the strength of the mechanical stimulus applied, the heart renews its activity and the intestine reestablishes its normal muscular contractions. If acetylcholine is involved in the transmission of nervous impulses to this organ, it should be possible to obtain some evidence of the action of this substance and other substances with which it has been said to be associated, when they are administered to this animal.

Methods

Daphnia magna young, when in their second instar, were used exclusively for the experimental work. The animals at this stage measure about 1 mm. in length, they are more transparent than the adult individuals and hence the changes produced in the course of the experimentation can be easily observed under the microscope. The mothers from

which the young were obtained for the experiments were reared at 25° C. in bottles containing the standard amount of the culture medium (Banta, 1921). The daily examination of the animals and the other methods employed in rearing the organisms in the laboratory (Obreshkove, 1930) enabled us at all times in the course of the experimental work to secure animals which were of the same age. A careful selection of the animals was necessary, because of the endeavor which was made to measure the period which elapsed between the addition of the particular chemical substances under investigation and the characteristic changes which they produced. The chemical substances employed were acetylcholine chloride, physostigmine (eserine) and atropine. Care was taken to use freshly diluted chemicals. The acetylcholine was adjusted to pH 5.7.

The animals were subjected to experimentation separately. A single individual was transferred to a micro culture slide with polished spherical concavity 18 mm. in diameter and approximately 3 mm. deep. The culture medium surrounding the animal was removed and immediately after this there was added the chemical substance whose action on the animal was to be studied. The amount of solution employed in each depression slide was kept the same and in each case it was just sufficient to cover the animal without permitting it to carry on extensive locomotive movements. This procedure enabled us to make continuous observations on a single individual under the microscope. The animal is seen at all times to ingest solid particles and fluid with which it comes in contact in the depression slide. With each opening of the mouth, a quick and powerful peristaltic wave of the esophagus forces the ingested material into the stomach, a process which can be easily observed under the microscope. Normally about 40 such peristaltic waves occur each minute. It is suggested, therefore, that the drugs employed in this work were administered orally.

Experimental Results

The intestine of untreated animals usually exhibits movements which are more or less rhythmic in nature. There is a gentle surging back and forth of the nutritive material and only when the animal is in the act of evacuating the contents of the intestine is one able to observe peristaltic and antiperistaltic waves in the musculature of the organ itself. At such times the forward peristalsis becomes more noticeable than the reverse peristalsis, the anus opens and the animal excretes only a small portion of the intestinal contents. This act is repeated at irregular intervals which vary from 30 seconds to more than 1 minute in some cases. At no

time, however, is the intestine entirely empty, for in the depression slide the animal is continuously reëngulfing the materials which it has evacuated.

The Action of Acetylcholine

When a *Daphnia magna* young is treated with acetylcholine, a very distinct change occurs in the intestine. The muscular peristaltic and antiperistaltic contractions of the organ become extremely violent and when stronger solutions are employed the entire contents of the intestine are emptied in a little more than a minute. The time which elapses between the application of the drug and the appearance of the first vigorous muscular contraction varies very definitely with the concentration of the drug employed. From an inspection of Table I it is seen that with acetylcholine 1×10^{-2} this occurs on the average in less than 20 seconds and with acetylcholine 1×10^{-3} this period is increased to 27.4 seconds. There is not a gradual development in the establishment of the violent intestinal activity. When the drug becomes effective, it exhibits its effectiveness to the fullest extent with an abrupt initial powerful contractile wave of considerable amplitude. After treatment with acetylcholine 1×10^{-3} and subsequent transference to water, vigorous forward and reverse peristalsis will continue in some cases for as long as 20 or 30 minutes. Acetylcholine 1×10^{-2} with lapse of time produces high intestinal tone and contracture.

When *Daphnia magna* young are treated with acetylcholine 1×10^{-4}, the time which elapses between the addition of the drug and the first appearance of the characteristic effect produced is on the average 10.7 minutes for the group of experiments presented here (Table I, column 3). With further dilution of the drug this period becomes longer. With acetylcholine 1×10^{-7} the time varies from 50 to 137 minutes (Table I, column 4), showing a definite and considerable increase over the time of reaction obtained with the higher concentrations of the drug.

The Action of Atropine

Atropine was found to antagonize the action of acetylcholine. When *Daphnia magna* are treated with acetylcholine until the characteristic powerful action of the intestine is established and then the solution is replaced by atropine, the effects of acetylcholine are quickly abolished. The powerful contractions, which would otherwise persist for many minutes, not only disappear but in many individuals after the atropine has become fully effective there is no longer any evidence of intestinal muscular contractions. Atropine 10^{-2} abolishes the effect of acetylcholine of

the same concentration in less than 20 seconds, but atropine was found to be effective even in dilutions of 1×10^{-9}. The range of effectiveness beyond this concentration of the drug was not investigated. The results obtained with acetylcholine 1×10^{-3} and atropine 1×10^{-5} are shown in Table II. The rapidity with which acetylcholine 1×10^{-3} produced its characteristic action on the intestine is shown here to be no different from that previously recorded in this paper (Table I). Atropine 1×10^{-5}, on the other hand, repeatedly abolished the effects of acetylcholine within 20 to 52 seconds. Table II also shows that following the abolishing of the powerful intestinal contractions by atropine, a stronger solution of

TABLE I

Onset of vigorous intestinal contractions in *Daphnia magna* after treatment with acetylcholine of various concentrations. The time of action is expressed in seconds or minutes and represents the period elapsing from the addition of the drug to the appearance of the characteristic effect.

Acetylcholine 1×10^{-3}	Acetylcholine 1×10^{-3}	Acetylcholine 1×10^{-4}	Acetylcholine 1×10^{-7}
seconds	*seconds*	*minutes*	*minutes*
20	25	10.8	119
20	35	8.2	137
22	40	8.2	123
19	30	14.4	125
22	30	12.3	74
15	20	9.3	113
16	22	11.7	110
17	25	12.2	124
19	20	9.3	50
24	27	10.8	69
Average 19.4	27.4	10.7	104.4

acetylcholine (1×10^{-2}) reëstablished the previous effect of acetylcholine, the average time for this being 21.8 seconds—a reaction time characteristic for this concentration of the drug (compare with Table I).

Acetylcholine and atropine of the dilutions employed in this work produced no lethal effect on the animals. Likewise atropine, when it was repeatedly administered to the same individual after treatments with acetylcholine, had no paralytic effect on the musculature of the intestine. To test this, a single individual was subjected to experimentation in the following way. The animal was treated with acetylcholine 1×10^{-2}. Immediately after the appearance of strong intestinal contractions, the drug was removed and replaced with atropine 1×10^{-5}. After the abolishing of the muscular contractions, the animal was again treated with acetylcholine and then atropine of the same dilutions as pre-

TABLE II

Onset of vigorous intestinal contractions in *Daphnia magna* after treatment with acetylcholine; the time of abolishing the acetylcholine effect by atropine; and the time of reestablishment of strong contractions by acetylcholine following atropine.

Acetylcholine 1×10^{-5}	Atropine 1×10^{-5}	Acetylcholine 1×10^{-7}
Time of action	Time of abolishing acetylcholine effect	Time of action of acetylcholine 10^{-7} after atropine
seconds	*seconds*	*seconds*
29	30	20
26	30	25
25	40	22
26	52	18
34	22	35
38	36	32
22	38	20
38	20	18
24	38	20
32	34	22
28	35	18
34	42	18
34	38	20
43	42	20
22	40	19
Average 30.3	35.8	21.8

TABLE III

The effects of repeated treatment of a single *Daphnia magna* with acetylcholine and atropine at regular intervals of a few seconds.

Acetylcholine 1×10^{-5}	Atropine 1×10^{-5}
Time of action	Time of abolishing acetylcholine effect
seconds	*seconds*
20	90
20	110
18	80
14	80
16	95
13	70
14	110
14	80
28	52
18	85
Average 17.7	85.2

viously employed. This procedure was repeated on the same individual for ten times and the results which were obtained are shown on Table III. It is evident from an inspection of the table that the drugs continued after each application to produce their characteristic effects. Atropine 1×10^{-5} under the conditions employed in this set of experiments required on the average 85.2 seconds to produce its characteristic effect, in comparison with 35.8 seconds (Table II), which was required for the drug of this dilution to block the effect of acetylcholine 1×10^{-3}. This difference in the reactivity is apparently due to the fact that the treatment with atropine in this particular set of experiments was preceded by a stronger solution of acetylcholine (1×10^{-2}) than heretofore employed in studying the antagonistic effect of atropine.

TABLE IV

Onset of vigorous intestinal contractions in *Daphnia magna* after treatment with acetylcholine (1×10^{-7}) following the administration of physostigmine (1×10^{-4} for 2 minutes) and the action of physostigmine 1×10^{-4} when administered alone.

Time of action of acetylcholine 1×10^{-7} after eserinization	Time of action of physostigmine 1×10^{-4}
seconds	*minutes*
45	9.9
47	10.0
52	10.2
58	10.0
41	9.7
55	10.3
70	9.4
37	10.1
42	9.7
30	9.6
51	9.9
41	10.4
97	10.7
52	9.5
34	9.5
Average 50.1	9.9

THE ACTION OF PHYSOSTIGMINE

Physostigmine (eserine) causes in *Daphnia magna* intensification and prolongation of the effects of acetylcholine. Likewise, after eserinization of animals, the acetylcholine becomes effective on the intestine in a shorter period of time. Fifteen animals which were treated with physostigmine 1×10^{-4} for 2 minutes and then with acetylcholine 1×10^{-7} yielded results which are shown in Table IV. The reaction time for eserinized individuals in the production of vigorous muscular contrac-

tions when treated with acetylcholine 1×10^{-7} is shown to be on the average 50.1 seconds as compared with 104.4 minutes when acetylcholine of the same concentration is employed alone (see Table I).

The intestine of *Daphnia magna* responds to a treatment of physostigmine when employed alone in the same way as it does to acetylcholine. When 15 animals were treated with physostigmine 1×10^{-4}, vigorous intestinal contractions appeared in about 10 minutes (Table IV, column 2). This relatively strong concentration of physostigmine was employed because the utilization of this strength revealed certain manifestations in the course of the action of the chemical substance which were not observed when higher dilutions were employed. The animal under the influence of the drug becomes immediately immobile. The wall of the intestine becomes opaque due to an extreme contraction of the muscular fibers and the intestine enters into a state of contracture. After 2 or 3 minutes the organism gradually begins to recover its normal swimming movements and the intestinal wall commences to reëstablish its normal state. In time there appear extremely powerful intestinal contractions. These contractions with lapsed time become more intensified and persist for a considerably longer period than when acetylcholine alone is administered to the animals. This period was often observed to extend over one hour after the drug is replaced by water.

Discussion

The action of acetylcholine, atropine and physostigmine on the intestine of *Daphnia magna* is such that it strongly suggests the possibility that this organ is controlled by cholinergic nerves. Acetylcholine, when applied in the concentrations employed in this work, was shown to intensify the intestinal activity. This action of acetylcholine was shown to be antagonized by atropine and augmented and prolonged by physostigmine. These and other observations recorded in this paper are in accord with the rôle which has been ascribed to these substances in physiological processes where nervous impulses are involved and where acetylcholine is believed to act as a transmitter of nervous impulses.

The sudden appearance of vigorous muscular contractions of the intestine under the influence of acetylcholine have enabled us to obtain certain data pertaining to the time which elapses between the application of the chemical substance and the onset of the specific effect produced. It is of considerable interest and importance to note that whereas acetylcholine in concentrations of 1×10^{-2} and 1×10^{-3} produces vigorous intestinal contractions in less than 30 seconds, with further dilution of the drug this period is considerably prolonged before the accelerating

response of the intestine to acetylcholine is noted. With acetylcholine 1×10^{-4} the period becomes, on the average, 10.7 minutes and with acetylcholine 1×10^{-7} the time which elapsed between the addition of the chemical substance and the appearance of the characteristic response was shown to be on the average 104.4 minutes. Latent periods of such extreme magnitudes are not in accordance with our present knowledge pertaining to the action of chemical substances which are thought to act as chemical transmitters of nervous impulses.

In view of the observation recorded in this paper it may be assumed that the effectiveness of acetylcholine is dependent on the rate of penetration and diffusion of the drug to the site of action, and on the rate of destruction. Acetylcholine 1×10^{-7}, however, when preceded by physostigmine 1×10^{-4} produces vigorous intestinal contractions in *Daphnia magna* in less than one minute. This indicates that acetylcholine of this relatively weak concentration reaches the site of action quickly and that the rate of penetration and the rate of diffusion in this particular instance are not primarily factors. However, it is possible that the rapid destruction of the acetylcholine when unprotected by physostigmine is responsible for the long delays preceding the onset of its characteristic action.

Artemov and Mitropolitanskaja (1938) have demonstrated the presence in whole *Daphnia* of an acetylcholine-like substance. As yet, however, no one has undertaken to demonstrate the presence or absence of choline esterase in this group of animals. The questions of how acetylcholine, if present in *Daphnia magna*, is bound in the tissues and how it is protected must wait further investigations before they are answered.

Summary

1. Acetylcholine produces in *Daphnia magna* vigorous intestinal contractions which persist for some time after they are established.

2. The period which elapses between the addition of the acetylcholine and the onset of the characteristic effect is definitely dependent on the concentration of the drug employed.

3. Atropine blocks the action of acetylcholine.

4. Physostigmine causes intensification and prolongation of the effects of acetylcholine.

5. Acetylcholine, when it is preceded by physostigmine, causes in *Daphnia magna* a considerable reduction in the time which elapses between the administration of the drug and the appearance of the vigorous intestinal contractions.

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VITAL STAINING OF THE CENTRIFUGED ARBACIA PUNCTULATA EGG

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The stratification and parts of the *Arbacia punctulata* egg obtained by centrifugal force are shown in Plate I (from E. B. Harvey, 1936). The size of the parts and degree of stratification varies with the centrifugal force; the greater the force, the larger the red half and the less marked the stratification (E. B. Harvey, 1941). In any experimental work with the halves and quarters, it is of importance to know exactly what materials are present. This is best done by the use of vital dyes which stain the different materials differentially.

Table I contains a list of vital dyes used, arranged alphabetically, and the effect of each dye on the various materials in the egg. In all cases, the egg was viable after staining, since it could be fertilized and at least begin development. Different brands of the same dye have been found in some cases to differ considerably both in staining capacity and in toxicity. In general, the dyes put out by the National Medicinal Products or the National Aniline and Chemical Co. gave the best results, but for Nile blue sulphate and thionin, Grübler's were better.

It was found better to stain the eggs first by allowing them to stand about one-half hour in a dilute solution of the dye in sea water, and then centrifuge them, because the mitochondrial layer disappears in 5–10 minutes after centrifuging and it often takes longer than that for the dye to be taken up by a centrifuged egg. No accurate measure of the amount of dye used was made, but it was soon learned how deeply the sea water should be tinged for the dye to be efficacious but not toxic. Some of the dyes are readily soluble in sea water, others (Bismarck brown, neutral red, Nile blue, safranin O, thionin) must be dissolved in distilled water and a drop of this added to the sea water; some vital dyes (e.g. cresyl violet, Victoria blue) were found not to be sufficiently soluble even in distilled water. No acid dye was found to enter the cell.

The *jelly* forms a layer around the egg which in *Arbacia punctulata* is 20–30 μ thick. It is invisible under the microscope unless outlined by particles of India ink or stained, since it is of the same refractive index as the sea water. When it is present, the eggs are well separated from each other; when the eggs are contiguous, it means that the jelly

has disappeared, and the eggs are then usually not in optimum condition. The jelly is destroyed by X-rays or by a small amount of acid in the sea water (1 drop of N/10 HCl + 50 cc. of sea water). It is sometimes centrifuged off while the eggs are rotating, though it may remain,

TABLE I

Arbacia punctulata. Vital dyes.

Dye	Jelly	Oil	Clear Layer	Mitochondria	Yolk	Pigment	Remarks
Bismarck brown	0	0	Yellow (upper part more intense)	Yellow	Yellow	Brown	Slightly soluble in sea water
Brilliant cresyl blue	0	0	0	0	Blue	Blue	Very innocuous
Chrysoidin	0	0	Light yellow (upper part more intense)	Light yellow	Yellow	Reddish brown	
Gentian violet	0	0	0	Purple	0	0	
Janus dark blue B	Purple	0	0	0	0	0	
Janus green (=diazin green)	Purple	0	0	Blue	0	0	Rather toxic
Methyl green	0	0	0	Purple	0	0	
Methyl violet	0	0	Upper part violet	Purple	Purple (later)	Purple (later)	
Methylene blue	0	0	0	0	Blue	Blue	Very innocuous
Neutral red	0	0	Pinkish yellow (lower part more intense)	Pinkish yellow	Brick red	Blood red, almost black	Slightly soluble in sea water
Nile blue sulphate	0	0	Light blue (upper part more intense)	Light blue	Blue	Bluish brown to blue black	Slightly soluble in sea water
Rhodamine	0	0	Pink (upper part more intense)	Pink	Pink	Deep red	Very innocuous
Safranin O	Yellow (few cases)	0	0	Pink (after 1–2 hours)	0	Blood red	Not soluble in sea water
Thionin	Pinkish (few cases)	0	0	Lavender (few cases)	0	0	Not soluble in sea water
Toluidin blue	Pinkish lavender	0	Pinkish lavender	Lavender	Lavender	Purple to blue black	More intense if stained after cent.

somewhat elongate, on well centrifuged elongate eggs, or even around the two separated half-eggs when close together, or it may remain around one half-egg. It is best to determine its reaction to dyes on uncentrifuged eggs. The jelly stains *purple* with *Janus green* and *Janus dark blue B*, and *pinkish lavender* with *toluidin blue;* in a few cases it stained *yellow* with *safranin O*, and *pinkish* with *thionin*.

The *oil* cap is not stained by any of the vital dyes. A slight tinge of color was observed in some cases, e.g. with Bismarck brown, chrysoidin and Nile blue, but it is probable that the slight color was in the matrix and not in the oil drops themselves.

The *nucleus* is not stained by any of the vital dyes.

It has been stated that the *clear layer* does not stain in the living egg (Lucké, 1925), and this is certainly true of many dyes. There is no doubt, however, that some dyes do stain the clear layer, not very intensely, while the egg is still living, as could be told by its subsequent development after fertilization. A comparison of the stained egg alongside a control egg in fresh sea water showed whether the clear layer was really stained. The clear layer stains *yellow* with *Bismarck brown* and *chrysoidin, blue* with *Nile blue, pink* with *rhodamine, pinkish yellow* with *neutral red* and *pinkish lavender* with *toluidin blue*. With some dyes there is a decided difference in the intensity of the stain in the upper and lower portions of the clear layer, indicating a stratification of materials within the layer. With Bismarck brown and chrysoidin, which in general act similarly, Nile blue and rhodamine, the upper portion of the clear layer stains more intensely. With neutral red, the lower portion stains more intensely. With *methyl violet,* only the upper portion stains (*violet*). This difference in different regions of the clear layer is more marked when the eggs are stained first and then centrifuged. Although the clear layer is optically empty in the living unstained egg, and no granules can be distinguished in the unstained or vitally stained egg, nevertheless in fixed material, this layer is filled with very fine granules, deeply staining (blue) with Heidenhain's iron hematoxylin (E. B. Harvey, 1940).

The best *mitochondrial* stain is *methyl green,* which stains the mitochondria *purple* and stains no other granules, so that the mitochondria appear as a purple band across the egg. *Gentian violet* also stains the mitochondria differentially (*purple*). *Methyl violet* stains the mitochondria *purple,* like methyl green, but it stains other granules as well. It may be that the purple stain of the methyl green is due to a contamination of this dye with methyl violet or crystal violet, but every brand of methyl green tried has given the same result. *Janus green,* which has been advocated especially by Cowdry as a mitochondrial stain, stains the mitochondria *blue,* but all brands have been found rather toxic, some brands more so than others. *Safranin O* was found to stain the mitochondria *pink* after some time, and *thionin* in a few cases stained them *lavender.* Other dyes stained the mitochondria, but also stained, somewhat more intensely, the underlying yolk (see Table I). As mentioned above, the mitochondrial layer disappears 5–10 minutes after

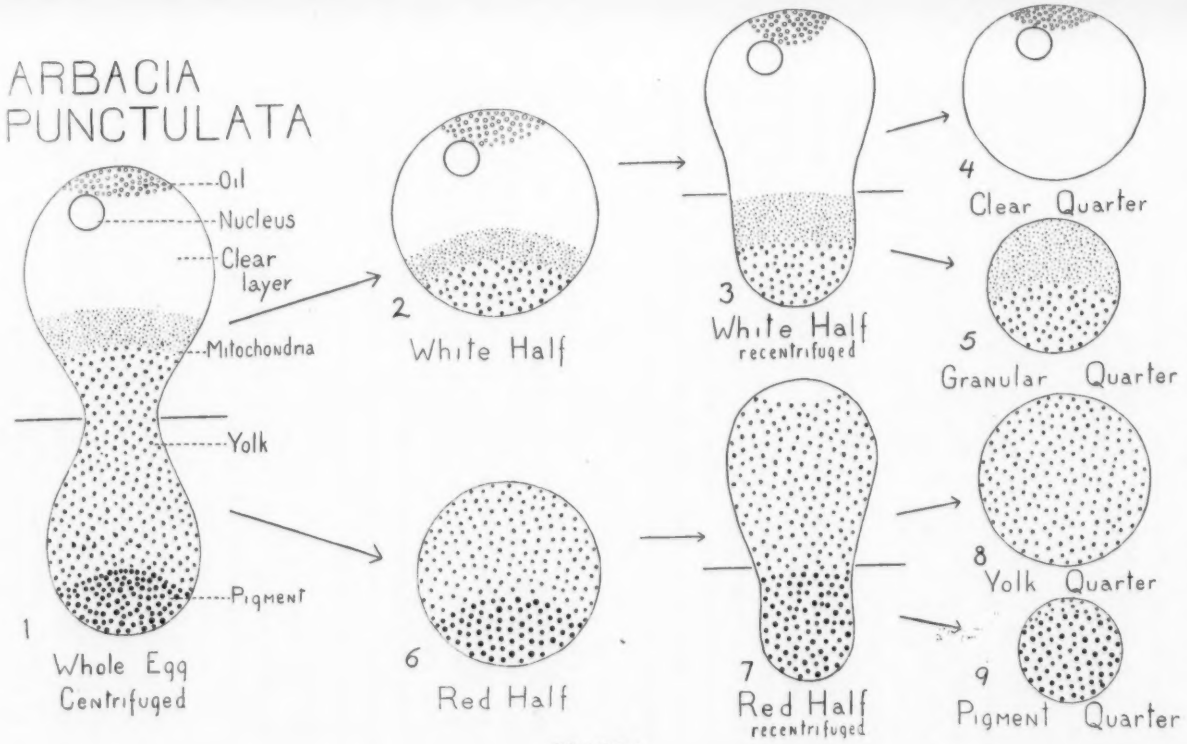

PLATE 1

The unfertilized egg of *Arbacia punctulata*, stratified by centrifugal force (about 3 minutes at 10,000 × g.), and the halves and quarters into which it breaks. The drawings are from camera lucida sketches and photographs, made as accurately as possible to scale. Magnified 500 ×.

The clear area in Fig. 7 at the centripetal pole is due to further packing of the granules with longer centrifuging.

removal from the centrifuge, so that observations on it must be made quickly. It is also the last layer to be formed in centrifuging, and in some batches of eggs is not at all sharply defined.

Yolk and *pigment* can be easily distinguished from each other in the unstained egg by the color. They are usually both stained with the same dye, the pigment more intensely and at first more reddish. They are stained *blue* with *brilliant cresyl blue* and *methylene blue,* which in general act alike, and *Nile blue; yellow-brown* with *Bismarck brown* and *chrysoidin; red* with *neutral red* and *rhodamine; lavender-purple* with *toluidin blue.* With *safranin O,* the pigment is stained *blood-red* and the yolk is unstained.

Considerable information as to the chemical structure of the materials in the egg might be obtained from a study of the dye reactions, since the chemical composition of the various dyes is known. For the chemistry and preparation of the dyes and other data, the reader is referred to Rowe's Color Index (1924), Schultz' Farbstofftabellen (1934) and Conn's Biological Stains (1940). For the rate of penetration of the dyes (into gelatin), see Möllendorff's excellent article in *Abderhalden Handbuch der biologischen Arbeitsmethoden,* Abt. V, Teil 2, Heft 2 (1921).

Summary

A table is given of the action of various vital dyes on the different materials in the centrifuged egg of *Arbacia punctulata.* The *jelly* surrounding the egg is stained with Janus green, Janus dark blue B, (purple) and toluidin blue (pinkish lavender). The *clear layer* is slightly stained with Bismarck brown and chrysoidin (yellow), Nile blue (blue), toluidin blue (pinkish lavender), rhodamine (pink), and neutral red (pinkish yellow). The *mitochondrial* layer is differentially stained with methyl green and gentian violet (purple) and Janus green (blue). *Yolk* and *pigment* are stained with brilliant cresyl blue, methylene blue and Nile blue (blue), toluidin blue (purple), rhodamine and neutral red (red), Bismarck brown and chrysoidin (yellow-brown). With safranin, the *pigment* is stained blood red, the yolk is unstained.

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ALLOMETRY IN NORMAL AND REGENERATING ANTENNAL SEGMENTS IN DAPHNIA

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The growth of a part in relation to that of the whole body in many animals follows the law of allometry and may be expressed by the equation

$$y = bx^{\alpha}$$

where y is the size of the part; x, the size of the whole; and b and α are constants (Huxley, 1932 and Huxley and Teissier, 1936). Regeneration has been "regarded as an acceleration of normal growth processes" (Przibram, 1919 and 1926). Regenerative growth might therefore also be expected to follow the law of allometry. Using the data of Zeleny (1908) on the gulf-weed crab, *Portunus sayi*, Huxley (1931) demonstrated that the above equation holds for the size of the chela in relation to the body as a whole and also for the amount of regeneration of the chela during any one instar in relation to the size of the animal during that instar. More recently others, especially Paulian (1938), have shown that in many arthropods the amount of regeneration of a part during any one instar in relation to the size of the body follows the law of allometry. Paulian has also pointed out that normal and regenerating antennae in *Gammarus pulex* and in *Carausius morosus* increase exponentially with time. Inasmuch as the relative growth equation is derivable on the assumption that the parts increase exponentially with time (Huxley, 1932 and Lumer, 1937), we may conclude that regeneration of the antennae in *Gammarus pulex* and in *Carausius morosus* also follows the law of allometry.

In the above cases we are dealing with regeneration that tends to be complete. Do these relations hold in a form where regeneration is not complete? In seeking a solution to the problem *Daphnia magna* is well suited as an experimental animal. After amputation of an antenna regeneration is limited to the restoration of the most proximal segment injured and the formation of setae. The amount of regeneration varies with the level of injury within the segment (Anderson, 1935).

The present study is a determination of the relations of the growth of normal and regenerating antennal segments to that of the animal as a whole in *Daphnia magna*.

Experimental Procedure

Females from a single clone of *Daphnia magna* Straus were used. The culture medium was pond water rich in organic matter. Individuals were isolated within six hours after their release from the mothers and placed in watch glasses with a few drops of culture medium. Just enough of a saturated solution of chloretone was added to make the animals immobile. Each animal was placed on its left side with the left antenna stretched out in front of the body. Those animals that were used to study the growth of normal segments were then drawn with the aid of camera lucida. After the drawings were made the animals were placed in individual vials containing about sixty cubic centimeters of fresh culture medium. In the case of those animals that

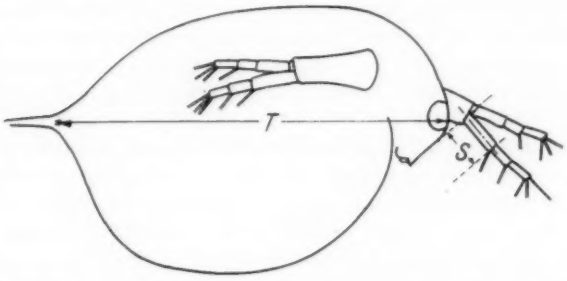

Fig. 1. Diagram showing the method of making measurements. *T*—total length, longest dimension of the body exclusive of the spine. *S*—segment length taken on the central axis of the segment.

were used to study regeneration, the first segment of the ventral ramus of the left antenna was severed by applying pressure with a needle which had been ground to a chisel edge. The level of amputation was varied in each instance. After the operation these animals were also placed in individual vials containing fresh culture medium. Several hours later the operated animals were again placed in watch glasses and immobilized with chloretone. They were then drawn in the same manner as were the unoperated animals. Care was taken to denote exactly the extent of the brown area just proximal to the level of amputation, for this is injured tissue that is cast off at the next molt (Anderson, 1935). After being drawn, they were replaced in their respective vials. From this point on both normal and operated animals were treated alike. Each animal was drawn during each successive instar up to and including the tenth. Inasmuch as the animals change in size only at ecdysis and im-

mediately thereafter (Agar, 1930), drawings were made at any time during the instar. Every time after an animal was drawn it was placed in fresh culture medium. The experiment was run at room temperature (18°–26° C.).

Measurements of the total length of the animals and the length of the antennal segments were made from the drawings. The total length of the animal was taken as the distance from the base of the spine to the most anterior point on the head. The length of the antennal segment was taken on the central axis of the segment. These measurements are illustrated in Fig. 1, and conform to those made by Anderson (1932, 1935) in other studies on *Daphnia magna*.

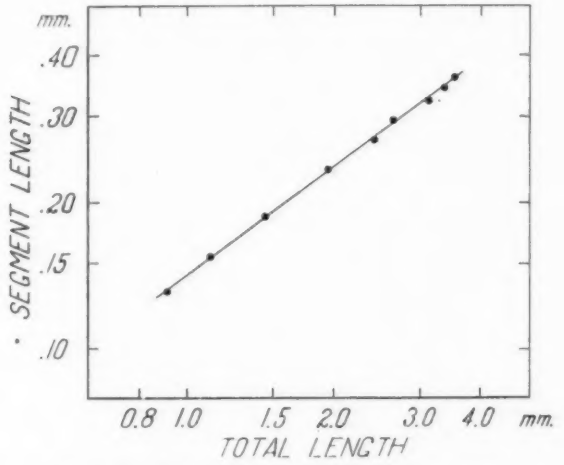

FIG. 2. Double logarithmic plot of the relations between the lengths of the normal segment and the total lengths during each of the first nine instars.

RELATIVE GROWTH OF THE NORMAL ANTENNAL SEGMENT

The relation of the logarithms of the mean lengths of the first segment of the ventral ramus of the left antenna to the logarithms of the mean total lengths of eighteen animals for the first nine instars is shown in Fig. 2. The points fall approximately in a straight line. We may therefore conclude that the law of allometry holds and the relation between the length of the segment and the total length may be expressed by the equation

$$y = bx^a \qquad (1)$$

where y is the length of the segment and x is the total length of the animal. The values of the constants b and α are 0.145 and 0.74, respectively. These were determined by the method of least squares. The value of α being less than unity indicates that the antennal segment grows at a lower rate than the body as represented by the total length. The antenna as a whole also grows more slowly than does the body as

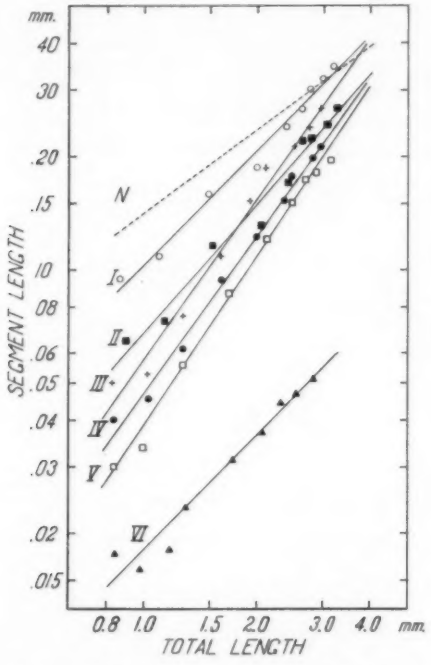

FIG. 3. Double logarithmic plots of the relations between the lengths of regenerating segments and the total lengths during each of the first nine instars for animals with antennae amputated at different levels. The level of injury is designated by the Roman numerals whose values are given in Table I. The dash line designated by N is that for the normal segments shown in Fig. 2.

is evidenced by the fact that the young at birth have antennae whose length is greater in proportion to the body than do the adults. The antennal segments, however, maintain the same proportions to each other throughout life.

Huxley (1931) has shown that the limbs of sheep grow less rapidly than the body after birth. This is also true for the macaques (Lumer

and Schultz, 1941) and probably for all mammals where the young at birth are able to run along with their mothers and perhaps for many other animals that depend on their means of locomotion for protection and/or food-getting.

Regeneration

The nature of regenerated antennae has been adequately described by others and need not be repeated here. Regeneration is limited to the restoration of the most proximal segment injured and the formation of new setae (see Anderson, 1935). The amount of regeneration is quantitatively related to the level of injury within the segment.

The operated animals were divided into six classes on the basis of the length of the intact portion of the segment during the latter part of the instar of amputation. The relations of the logarithms of the mean

Table I

The values of b and α for the relations of the length of the regenerating first segment of the ventral ramus of the left antenna to the total length of the animal.

Class	Level of Injury *	Number of Cases	b	α
I	0.076–0.110	8	0.103	1.01
II	0.060–0.070	7	0.068	1.14
III	0.046–0.053	7	0.058	1.42
IV	0.040–0.042	8	0.047	1.40
V	0.027–0.030	9	0.039	1.49
VI	0.010–0.023	7	0.018	1.02

* Length of the intact portion of the segment in millimeters during the instar of amputation. The values of b and α were determined by the method of least squares.

lengths of the amputated segments to the logarithms of the mean total length of the animals for the instar of amputation and the next eight instars for each class are shown in Fig. 3. While the points for any one class do not fall along a straight line as closely as do those for the relations in unoperated animals (Fig. 2), they do approximate a straight line. The law of allometry may be considered applicable and the relations can be expressed by the equation (1). The values of the constants are given in Table I.

The value of the constant α in the equation (1) is the ratio of the percentage increase of y to the percentage increase in x. Since x always represents the total length of the animals, both normal and operated, in the relations described above, the values of α are directly comparable. Examination of Fig. 3 and Table I shows that the value of α increases

as the level of injury reaches a lower point in the segment until a certain level is reached after which the value of α decreases. This relation is brought out graphically in Fig. 4. Another point worthy of note is that in Fig. 3 the curves with one exception tend to converge at a point where the total length would be about five millimeters, the maximum size that the animals reach. From this it is apparent that as long as the level of injury is above the critical level, the growth rate of the regenerating antennal segments is such that they approach the length of the normal segment simultaneously as full growth of the animals is attained.

Somewhat analogous results have been found by others. Zeleny (1905, 1909) found that the rate of regeneration of an organ in many

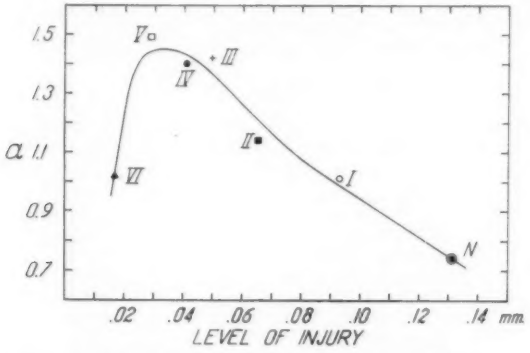

FIG. 4. The relation between the value of α in the equation

$$y = bx^{\alpha}$$

and the level of injury. The symbols correspond to those used in Fig. 3.

animals increases with the degree of injury up to an optimum, after which the rate decreases. Zeleny's work is not directly comparable inasmuch as he was concerned with the rate of regeneration of an organ when that one only was removed in comparison with the rate when several others were removed in addition. The results of Paulian (1938) are more directly comparable. He amputated the antennae of *Gammarus pulex* and *Carausius morosus,* and inspection of his figures (Figs. 14 and 15, pages 320 and 322) indicates that the rate of growth of the antennae increased as the level of amputation approached the proximal end. Whether or not a critical level might be reached beyond which the rate decreases, was not determined in his experiments. Further, the amputated antennae reach the size of the normal at different times, the time taken varies directly with the amount removed.

The Significance of the Constant b

The question of the biological significance of the constants b and α in the law of allometry have been subject to considerable discussion. Huxley (1932) and Needham (1934) have stated that the constant b is of little biological importance. The value of the constant b is that of y when $x = 1$. As a consequence, its value changes with the unit of measure employed while the actual relations of y and x remain the same. Again the unit chosen is usually such that b is an extrapolated value of y. Because of these arbitrary factors the significance of b has remained elusive. Recently Lumer, Anderson, and Hersh (1941) have pointed out that if b is to have biological significance, the unit of measure chosen should be one given by the organism. They suggest that the most satisfactory unit would be the size of a standard part at the beginning of a developmental period, but where this cannot readily be ascertained an approach to it could be made by taking the smallest value of the standard part given by the data as unity. In this way b would be an actual value of y. This is in line with the proposal of Huxley and Teissier (1936) that b should be called the "initial-growth index," for indeed that is what it becomes as far as the data are concerned when the above suggestions are followed. The constant α has presented no such difficulties. Since it is the ratio of the percentage growth rates of the parts y and x, it is constant regardless of the unit of measure used. α has therefore been considered of relatively greater importance than b.

Lumer, Anderson, and Hersh (1941) have shown how the constant b may be made a more tangible entity in that it can be given in terms of the organism. As such it has significance. The question still remains as to the degree of its importance. If the value of b, i.e., the initial ratio of y to x, could be altered experimentally, and if as a consequence the value of α would change, we could conclude that the value of b determines the value of α; b would then have a greater biological importance than heretofore supposed.

This is precisely what we have done in the experiments described in this paper. We have amputated the antenna and so reduced the length of the segment. The ratio of the intact portion of the segment during the instar of amputation to the total length of the animal is given by b, since the total length during that instar is approximately one millimeter and the millimeter is the unit of measure. Following amputation, the growth rate of the antennal segment is changed so that new values of α result. Further, as b decreases α increases until b reaches a particular value, after which α also decreases as is shown in Table I and Fig. 4. The constant b, in the sense in which we have employed it, serves as a measure of the conditions at the beginning of the developmental period,

and as these conditions differ, so also do the consequent rates of development as represented by the constant α.

Summary

The law of allometry

$$y = bx^{\alpha}$$

was found to be applicable to both normal and regenerating antennal segments in *Daphnia magna*.

The growth rate of the regenerating segments increases as the level of injury approaches the proximal end of the segment until a critical point is reached, after which the rate decreases. As long as the level of injury is distal to the critical level, the growth rate is such that the regenerating segments tend to approach the length of the normal segment simultaneously as full growth of the animals is attained.

The significance of the constant b in the law of allometry is discussed.

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EXPERIMENTAL CYTOLOGICAL EVIDENCE FOR AN OUTWARD SECRETION OF WATER BY THE NEPHRIC TUBULE OF THE CRAYFISH [1]

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Introduction

When a crayfish is in freshwater, its normal habitat, it does not drink, but water diffuses into its body through the gills (Maluf, 1937, 1940). An internal aqueous and saline steady state is maintained, in spite of a constant inward diffusion of water, because of the unvarying capacity of its kidneys to manufacture urine that is markedly hypotonic to the blood (Schlieper and Herrmann, 1930; Herrmann, 1931).

The concentration of chloride in the luminal fluid of the coelomosac and labyrinth of the nephron is equal to that in the blood, but that in the tubular fluid is markedly lower than that in the blood (Peters, 1935). The hypotonicity of the urine must therefore be due either to an active resorption of salts by the tubule or to an outward secretion of a hypotonic liquid by the tubule.

The tubule of a 35-gram animal is approximately 3 cm. long and about 2 mm. in greatest breadth.[2] The ventral, i.e., proximal, half of the tubule consists of flat cells without apical secretory globules. The dorsal, i.e. distal, coil is composed of relatively large columnar cells with a distinct mitochondrium and, generally, with large clear apical vacuoles which bulge into the lumen of the tubule (Maluf, 1939).

Because the nephron of the crayfish does not possess a tenuous syncytium, such as the glomerular capsule of the vertebrate nephron, filtration seems unlikely as a major process of urine-formation. Accordingly, this is an attempt to find whether the apical vacuoles of the distal coil of the tubule represent an outward secretion of water.

The experimental attack is partly based on the observation of Herrmann (1931) that, as the salinity of the external medium is raised, the rate of urinary flow falls and the osmotic pressure of the urine simul-

[1] This work was performed when the author was Johnston Research Scholar in the Department of Zoölogy, The Johns Hopkins University. To Professor S. O. Mast much obligation is due for numerous kindnesses.

[2] In the 1939 paper this was misprinted as "2 cm."

taneously increases. As shown by constancy in weight, the total quantity of water in the crayfish is the same in freshwater as in salinities up to 272 mM. NaCl per liter, which is initially hypertonic to the blood. This indicates that the decrease in the rate of urinary flow, with rising external salinity, is not due to a decrease in haemocoelic pressure which, assuming that filtration does occur, might cause a decrease in the rate of filtration. There is, furthermore, no apparent basis for the supposition that the haemocoelic pressure undergoes a localized fall in the vicinity of the kidneys as the salinity of the external medium is raised.

From the above it might be expected that, when the crayfish is in a medium in which inward diffusion of water can be only very small and in which the rate of urinary flow is accordingly depressed, the apical vacuoles of the nephric tubule will tend to disappear.

Methods

The test animals were immersed in 210 mM. NaCl per liter of freshwater, a solution in which they can remain vigorous indefinitely. Although this concentration is somewhat hypertonic to the blood at the outset (see Lienemann, 1938, for the normal osmotic pressure of the blood of *Cambarus clarkii*), some water, probably only a negligible quantity, diffuses inwardly because, as Herrmann (1931) showed, the osmotic pressure of the blood eventually exceeds that of the external medium. Parenthetically, the invariable hypertonicity of the blood, as compared with the external medium, is probably mainly because the urine is always hypotonic to the blood regardless of the osmotic pressure of the external medium (Herrmann). Integumental uptake of salt from the exterior is a relatively minor factor, as can be readily calculated (data of Lienemann, 1938, and Maluf, 1940).

At the end of one to several days the animals were sacrificed and their kidneys removed with minimum handling and fixed in unneutralized formol-sublimate for several hours, washed in running tap-water overnight, dehydrated with dioxane (50 per cent, 75 per cent, and two changes of 100 per cent), imbedded in paraffin with a melting point of about 49° C., sectioned 8 μ thick, and stained with eosin and methylene blue-borax.

Results

A seven-day stay of three animals in 210 mM. NaCl per liter abolished almost all the apical vacuoles from the cells of the distal coil of the nephric tubule (Fig. 1) whereas the majority of the corresponding cells of the three controls, which had been in freshwater, possessed large

apical vacuoles which bulged into the lumen of the tubule (Fig. 2). One of the test animals exhibited an exceptional number of vacuoles for an animal in 210 mM. NaCl but the vacuoles were small and scanty as compared with those of the controls. The photographs were taken from areas at random. The data were analyzed objectively as follows: In this experiment two to three slides were prepared containing serial sections of the pair of kidneys from each individual (16 slides in all); the labels were covered so as to remove every vestige of external identification; the slides were shuffled. The examination of each slide never exceeded one or two minutes and was made under low power (100 ×). In 15 slides out of 16, the identification of the series to which the preparation belonged (freshwater or 210 mM. NaCl) was correct. Measurements did not show a correlation between the height of the cells and the existence of apical vacuoles.

The experiment was repeated with six larger animals and a duration of three days. Here, too, the difference between the three test animals in 210 mM. NaCl per liter (Fig. 3) and the controls (Fig. 4) was pronounced. The objective analysis, identical with that above described, showed a correct identification of 20 slides out of 22. Here, too, extensive measurements indicated no correlation between the height of the cells and the presence of vacuoles. The three-day experiment was repeated with confirmatory results: the two test animals showing practically no apical vacuoles whereas the two controls displayed apical vacuoles in the majority of cells of the distal half of the tubule.

Even a 24-hour stay in 210 mM. NaCl produced a practically complete abolition of the apical vacuoles (Fig. 5) although the interior of the cells was considerably vacuolated. Nearly all of the corresponding cells of the controls in freshwater exhibited large clear apical vacuoles (Fig. 6). Figures 5 and 6 are at a lower magnification than the other photographs and thus exhibit a larger field. The objective analysis showed a correct identification of 8 slides out of 8. Subjection to 210 mM. NaCl for less than 24 hours was not attempted.

After vacuole-formation has presumably been practically abolished by an 168-hour stay in 210 mM. NaCl, the vacuoles reappear upon returning the crayfish to freshwater. In this experiment there were two tests and two controls.

The fact that a large fraction of the cells of the distal half of the tubule of the controls invariably exhibited large apical vacuoles in itself shows that the almost complete absence of such vacuoles in slightly hypertonic NaCl is not an artefact of histological technique. The distal half of the tubule of a live animal was dissected out of the kidney in

crayfish-saline.[3] Fragments teased out of this part of the tubule and suspended in a hanging drop of crayfish-saline on a coverslip, gave an ample picture of the vacuoles.

Historical Statement and Discussion

The nephric tubule of the decapod kidney was first discovered by Neuwyler (1841), who did not understand the function of the "green glands." Only within the present decade have we come to realize the importance of the crustacean kidney in the aqueous and ionic regulation of the bodily fluids. Grobben (1881) was the first to observe that the nephric tubules of freshwater Crustacea and Annelida are markedly longer than those of corresponding marine forms and that length of tubule is not correlated with bodily size. He did not theorize as to the significance of these facts but remarked that, "It therefore appears that the length of the urinary canal goes parallel with life in freshwater." Richard (1891) came to an identical conclusion with regard to copepod Crustacea. Rogenhofer (1905, 1909) confirmed Grobben and found that differences in the nephric dimensions of marine and freshwater Crustacea are not due to differences in cellular size. Rogenhofer failed to alter the length of the tubule of the freshwater isopod, *Asellus aquaticus*, in one generation by gradually bringing the isopod to a salinity of 2 per cent in one year. Della Valle (1893) believed that the differences

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PLATE I[4]

Explanation of Figures

Fig. 1. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for seven days. *ha*, haemocoele and blood-vessels; *LU*, lumen of tubule. Triple Mallory's; daylight bulb; Zeiss lens. Animal about 10 grams.

Fig. 2. Control to Fig. 1; animal in freshwater. *va*, large apical vacuoles. Animal about 10 grams.

Fig. 3. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for three days. Methylene blue-eosin; red filter; Zeiss lens. Animal about 30 grams.

Fig. 4. Control to Fig. 2; animal in freshwater. Animal about 30 grams.

Fig. 5. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for 24 hours. Methylene blue-eosin; red filter; Zeiss lens. Animal about 13 grams.

Fig. 6. Control to Fig. 5; animal in freshwater. Animal about 13 grams.

[3] The saline was based on the most acceptable data on the concentration of inorganic electrolytes in the blood of the crayfish (see Maluf, 1940, for references) and was as follows (g./l.): NaCl, 7.81; $CaCl_2$, 1.31; $MgCl_2$, 0.82; KCl, 0.70; buffered at pH 7.5 with 0.5 cc. M/5 Na_2HPO_4/NaH_2PO_4. A $\triangle$ of about 0.66° C. is assumed (see Lienemann, 1938, and Schlatter, 1941).

[4] The writer is much indebted to Dr. Charles E. Brambel, The Johns Hopkins University, for kind personal instruction in photomicrography.

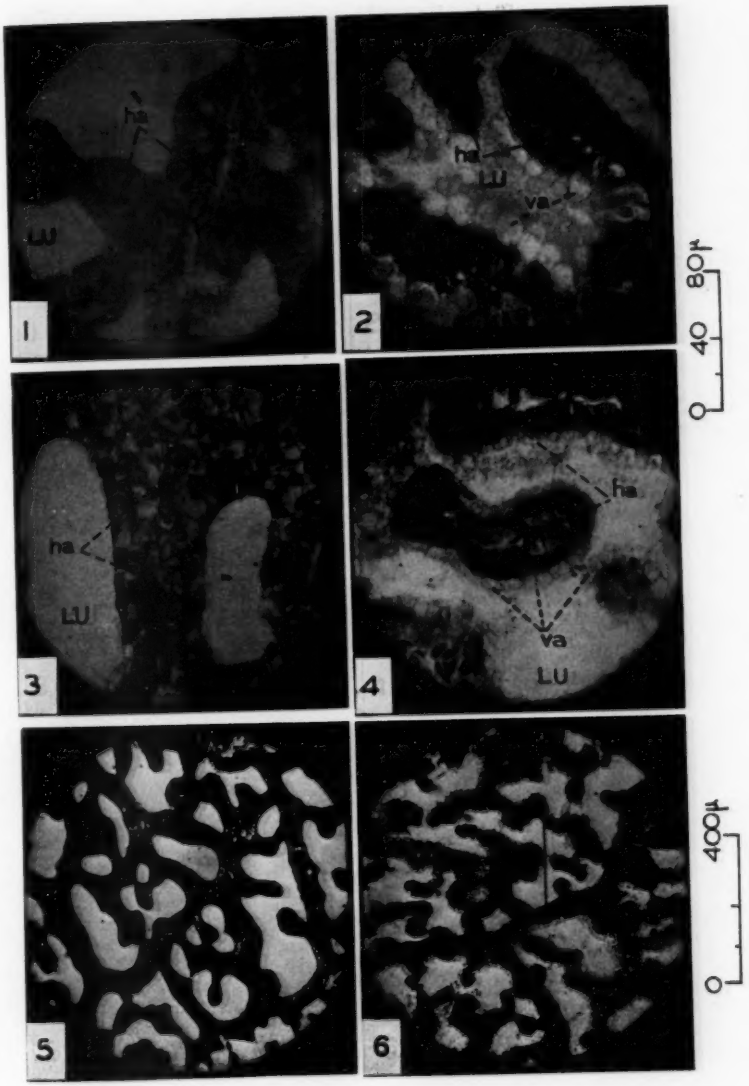

PLATE I[4]

in tubular length are of phylogenetical origin rather than a direct environmental effect. Marchal (1892) observed that the nephrons of the lobster and other marine decapods have no tubule. He suggested that the external medium,—freshwater and sea-water,—may be a determining factor but remarked that the estuarine crab, *Telphusa,* has no nephric tubule even though it frequents freshwater.

In 1930, Schlieper and Herrmann found that the urine of the crayfish is markedly hypotonic to the blood and that the urine of the shore-crab, *Carcinus maenas,* and of the estuarine crab, *Telphusa fluviatilis*—neither of which possess nephric tubules—is isotonic with the blood. They suggested that the nephric tubule is responsible for the hypotonic urine of the crayfish and that it acts by resorbing salts from a filtrate formed at the coelomosac. Herrmann (1931) and Peters (1935), in Schlieper's laboratory, demonstrated that the tubule is of paramount importance in osmoregulation. Peters suggested that the apical vacuoles of the distal coil may indicate a resorption of salts from lumen to blood. Peters' theory presupposes that a filtrate is formed somewhere in the nephron proximal to the tubule. Peters made the important discovery that only in the tubule is the concentration of chloride of the presumptive urine lower than that of the blood. His results do not show, however, in which part of the tubule this is true.

The facts in this paper suggest that the vacuoles represent an outward secretion of water in compensation for that which diffuses inwardly. Physiological data indicate that the crayfish nephron is paramountly if not entirely a secretory organ (Maluf, 1941). The hypotonic urine of this animal may thus be the result of an outward secretion of a liquid markedly hypotonic to the blood and the rate of water-secretion by the tubule may be determined by a hormone.

Summary

The majority of the cells of the distal half of the nephric tubule of the crayfish exhibit large, clear apical vacuoles at their luminal borders when the animal is in freshwater, its normal medium.

If the crayfish remains in a saline medium which is initially slightly hypertonic to the blood, for twenty-four hours or more, these vacuoles completely disappear. The condition is reversible upon return of the animal to freshwater. (The crayfish can maintain its vigor indefinitely in 210 mM. Nacl per liter, which is initially slightly hypertonic to the blood.)

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MICTURITION IN THE CRAYFISH AND FURTHER OBSERVATIONS ON THE ANATOMY OF THE NEPHRON OF THIS ANIMAL

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Preliminary to studies on renal function in the crayfish (Maluf, 1940, 1941*b*), it is necessary to know how urine is retained in the bladders and how discharged. Nothing has been indicated, until the present, as to how urine is retained. There is, furthermore, no adequate study of the anatomical features surrounding the urinary outlet of decapod Crustacea. As a result of this deficit, investigators of renal function in the crayfish have punctured the membranous operculum at the nephropore prior to collecting urine by suction (Marchal, 1892; Boivin, 1929; Herrmann, 1931; Scholles, 1933; Lienemann, 1938). It is not clear why the opercula were destroyed. From Marchal's diagrams it appears that removal of the opercula would tear the ureters and lead into the haemocoele and that, consequently, the urine would be contaminated with blood. Marchal and Boivin, however, stated that the liquid they collected was limpid, clear, and almost colorless and practically uncontaminated with blood. The chemical analyses of Herrmann, Scholles, and Lienemann show that the concentration of inorganic electrolytes in the liquid collected from the excretory orifices was markedly lower than in the blood. The fact that the distal portion of the bladder contacts the base of the excretory eminences at most of its circumference (Fig. 2, *B*) apparently explains how the urine collected by the afore-mentioned investigators did not contain an appreciable quantity of blood. The urine aspirated by Picken (1936), by piercing the operculum with a fine hypodermic needle, was doubtless, at times at least, notably contaminated with blood as shown by the strongly positive xanthoproteic reaction and by the large discrepancies, in this respect, with regard to the urine from both kidneys. Thus, in one instance, the urine from the right kidney gave a negative xanthoproteic test while that from the left gave a strong reaction. The writer found that urine collected from *Cambarus clarkii* by suction from intact nephropores invariably gave a weak xanthoproteic but a negative biuret reaction.

The review of Burian and Muth (1924) may leave one with the impression that the communication between the coelomosac and labyrinth "is closed by a sphincter muscle, and any passage of fluid from the labyrinth into the coelomic sac appears to be prevented by a valve-like arrangement of cells" (Picken, 1936). Examination of the literature left the writer dubious about the existence of a sphincter between the coelomosac and labyrinth. The present paper shows that, at least in *Cambarus clarkii*, there is no sphincter or valve between coelomosac and labyrinth or between nephric tubule and bladder.

Weismann (1874), Grobben (1881), Schlieper (1935), and Peters (1935) believed that a blood-ultrafiltrate is formed in the coelomosac. The writer has made a detailed histological examination of this part of the nephron to find out whether the histological facts support the hypothesis of filtration.

The results in this paper refer to *Cambarus clarkii*, the swamp crayfish.

Micturition

The Retention of Urine

Because the bladders are normally distended with urine and because urine only occasionally leaves the nephropores of undisturbed unheated animals seen under a microscope, urine must be adequately retained in the bladders. The volume of retained urine was sometimes as much as 4 per cent of the fresh weight of the animal.

On the ventral surface of the basal segment of each second antenna is the whitish excretory eminence (Fig. 5, *e*) in the central depression of which is a convex, finely corrugated, flexible, thin membrane, *o*, known as the operculum. Because the operculum does not cover anything external, the name is inaccurate. The convexity of the operculum is maintained by blood-pressure, as the opercula invariably collapse after thoroughly bleeding the animal. In contrast to the rest of the excretory eminence, the operculum is very sensitive to contact as shown by the resulting generalised motor response. The operculum is invaginated at its anterior border, thus forming a narrow crescentic slit (Figs. 2, 3, 5, and 6, *a*) which is the actual excretory orifice, or nephropore. The invagination proceeds at a sharp angle posteriorly, forming the short flat ureter (Figs. 2 and 3, *ur*).

The rounded flexible contour of the operculum is inessential. An animal with both opercula damaged by puncture was under observation for about a month, at the end of which time its opercula were still collapsed. The ureters, however, were not damaged, as shown by subsequent dissection.

The ureter (Figs. 2 and 3, *ur*) is short, dorso-ventrally flattened, and parallel to the operculum. Fine spindle-shaped fibers (Figs. 2 and 3, *f'*) containing elongate nuclei (13 μ long in crayfish-saline) extend from the dorsal wall of the ureter to the basal margins of the excretory eminence. With care, the whole dorsal wall of the ureter, including the fibers, may be dissected and mounted.

The fibers are unstriated (observed at 970 $\times$ while in fresh saline or after being fixed in formalin and stained with haematoxylin or Wright's) and are apparently identical with those which stretch between the distal extremity of the bladder and the integument (Fig. 2, *f*, *f''*). These ureteral fibers apparently act as a sphincter and their discovery answers the question as to how urine is retained in the bladders. Because a gentle outflow of urine has been seen in deviscerated inverted animals, the bladder must be elastic and the ureteral sphincter evidently normally retains urine in the bladder by tonic contraction.

Similar fibers occur, circularly arranged in considerable numbers, on the haemocoelic surface of the most proximal portion of the bladder, to a very much slighter degree on the main body of the bladder, and also on the main stem of the renal artery. Spindle-shaped unstriated fibers have been observed on the bladder of the American lobster by Waite (1899).

PLATE I

FIG. 1. Dorsal aspect of the opening into the left second antenna and surrounding exoskeleton, showing the distal portion of the bladder wedged between the proximal antennal muscles. *am*, articular membrane between antenna and cephalothorax; *B*, distal portion of bladder; *bas*, basipodite; *c*, lateral wall of the cephalothorax; *comp*, compressor muscles of the antenna; *cox*, coxopodite; dep_{1-4}, depressor branches of the antenna; *lev*, levator muscle of the antenna; *prom*, promotor muscle of the antenna; *rem*, remotor muscle of the antenna; *s*, sternum.

FIG. 2. Sagittal section through the distal portion of the bladder, ureter, and nephropore. *B*, distal portion of the bladder; *c*, connective tissue; *f*, *f'*, *f''*, unstriated fibers; *f'*, ureteral sphincter; *n*, nephropore; *o*, operculum; *s*, coxopodite; *ur*, ureter.

FIG. 3. Dorsal aspect of the ureter and the depression of the coxopodite which corresponds to the eminence of the ventral aspect. *a*, nephropore, shown in broken lines because it is ventral to the ureter; *f'*, ureteral syncytium; *ur*, ureter.

FIG. 4. Dorsal aspect of the brain. *a*, nerve-stems passing into the lumen of the second antenna; c_1, c_2, individual nerve-fibers issuing from the roots of the former; *lc*, longitudinal connectives; *m*, median nerve; *oc*, oculomotor nerve; *op*, optic nerve; *P*, protocerebrum; *T*, tritocerebrum; *te*, "tegumentary" nerves. The root of the nerve to the first antenna issues from the ventral surface of the brain and is thus not shown here.

FIG. 5. Ventral aspect of the region surrounding the right nephropore. *a*, crescentic nephropore; *e*, excretory eminence of the basal segment of the second antenna; *o*, operculum; *s*, coxopodite; *u*, droplet of urine.

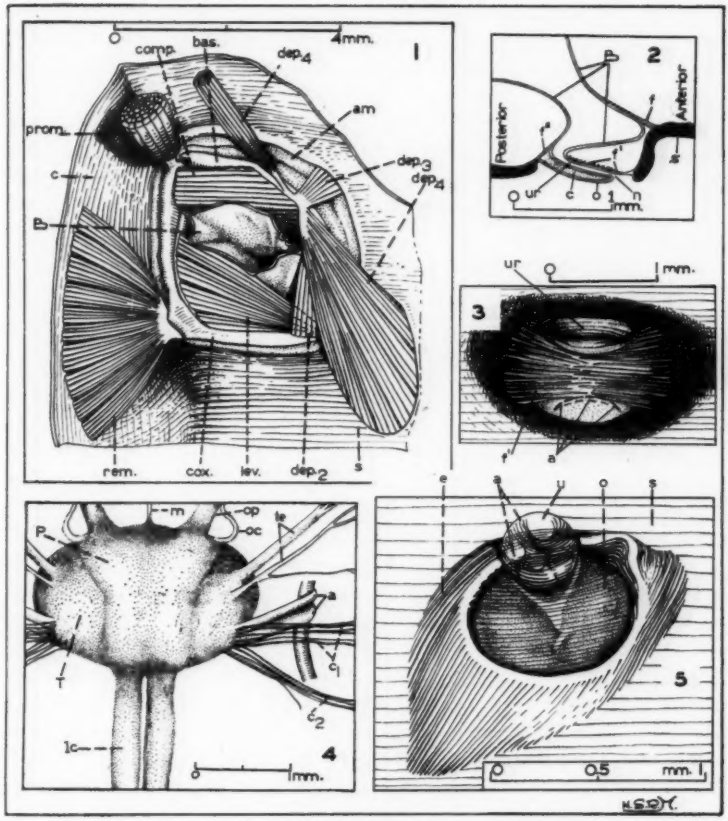

PLATE I

(All figures refer to *Cambarus clarkii;* the animals of Figs. 1, 4, and 5 measured about 7.5 cm. from rostrum to end of telson.)

In spite of numerous careful dissections, the writer has not been able to find any fibers inserting on the operculum. This conforms with Marchal's (1892) observations on the crab, *Maia*. Schmidt (1915), who gave a comprehensive and well-illustrated account of the somatic musculature of the European crayfish, did not mention any special muscles of micturition. The region between the operculum (Fig. 2, *o*) and the ureter, *ur*, is occupied by connective tissue and does not contain spindle-fibers.

At the posterior margin of the excretory eminence the ureter bends sharply anteriorly, enlarges in girth, and continues as the bladder (Fig. 2, *B*). Upon emerging from the excretory eminence (Fig. 2), the bladder passes through a mass of antennal muscles (Fig. 1).

The Expulsion of Urine

The animal was drained of moisture, water was sucked from the branchial chambers, and the anterior border of the chambers plugged with absorbent cotton-wool to prevent remaining water from flowing over the opercula. The opercula were observed under magnifications of 22.5 and 112.5 ×.

The outflow of urine in air occurs anteriad, i.e. in the plane of the ureter. At times the urine issues from the orifice for a short distance and is then sucked back. Slight pressure on the operculum with a blunt instrument frequently induces urinary outflow. A pipette of suitable size and carrying suction (about 10 mm. Hg) may produce urination even for some time after the use of suction. The urine issues as a series of generally spherical droplets. The suction does not injure the operculum. The latter does not undergo movement except for a scarcely perceptible motion only as the urinary droplet attains maximal size. This is doubtless a passive effect. Marchal (1892) stated that, in the crab *Maia*, movements of the opercula accompany the discharge of urine; it is probable that in *Maia*, too, the motion is passive. Marchal stated that muscles do not insert on the operculum of *Maia*. The writer confirms this for the crayfish.

Not infrequently, while the animal was held dorsum down and both nephropores were apparent, fine jets of urine abruptly spurted from both orifices sometimes to a distance of a foot or more. Every jet consisted of droplets in quick succession. On one occasion the occurrence was especially striking in that a series of jets to at least a foot followed one another rapidly. Although the spurts from both nephropores generally were not entirely simultaneous, the writer cannot recollect any instance in which urine spurted from one nephropore and not from the other

within a brief interval of time. Such powerful and sudden jets cannot be accounted for by the very sparsely distributed unstriated fibers of the bladder. Other decapods act similarly. In a single instance the estuarine crab, *Callinectes hastatus,* immediately on being grasped spurted urine to a distance of about 9 cm. from both nephropores simultaneously. Marchal (1892) noted a distance of 2 cm. from a shrimp and Herrick (1909) "an inch or more" from the American lobster on being held. Herrick ascribed the phenomenon to contractility of the bladder but evidently made no observations to support this supposition.

Whether the sparsely scattered vesicular fibers contribute to the discharge of urine is still unknown. The bladder was subjected to electrical induction shocks of high and low frequency, led through fine Ag–AgCl electrodes, both while distended with urine *in situ* and when isolated and under slight stretch in the longitudinal or in the transverse direction between two points in crayfish-saline. Contraction was never observed even under a magnification of 22.5 ×. The induction shocks were capable of causing cardiac tetanus, contraction of the dorso-anterior and -posterior dilators of the crop-gizzard, of the dorso-posterior longitudinal muscles of the crop-gizzard, and of the intact and isolated intestine, and abduction and adduction of the claw of the cheliped. Electrical stimulation of the bladder frequently produced strong generalised somatic muscular contraction; abrupt flexion of the abdomen and contraction of the homolateral remotor of the second antenna (Fig. 1, *rem.*) were among the main effects. Because the latter muscle is contiguous with the latero-ventral surface of the bladder, its contraction generally falsely suggested contraction of the bladder. Marchal (1892) briefly stated that he was unable to elicit contraction of the bladder of *Maia* by electrical stimulation.

Doubtless the major factor in the expulsion of urine is pressure exerted on the bladder by the blood and crop-gizzard. The nephropores, of animals drained from moisture, were often observed to remain dry for forty minutes or more. The injection of 1 to 1.5 cc. of saline into the haemocoele, between the chelipeds, i.e., in the vicinity of the bladders and crop-gizzard, invariably resulted in an immediate outflow of urine from both nephropores simultaneously. Merely puncturing the integument did not produce effects. If urination was occurring slowly, the injection of 1 to 1.5 cc. of saline resulted in a marked increase in the rate of outflow. It is conceivable that in some instances both bladders may be entirely collapsed; urination then would not be expected even upon the injection of any amount of liquid. Bilateral compression of the integument lateral to the bladders often produced an outflow of urine or an increase in the rate of flow. The large crop-gizzard is partly

wedged between the upper surfaces of the bladders. As direct mechanical pressure on the bladders results in their collapse and in the expulsion of urine, the movements of the crop-gizzard must be a factor in urination.

Innervation

Probably because the kidney and bladder are organs of the second antennal somite, all nerve-fibers to the bladder issue from the tritocerebral lobe of the brain (Fig. 4, *T*). The anterior component of the tegumentary nerves, *te,* sends a branch to the integument beneath the labyrinth; the posterior component sends branches to some of the proximal muscles of the second antenna. About nine fibers issue in the anterior cluster, c_1, which arises from the base of the root of the main antennal nerve-trunks, *a*. Fibers from c_1 innervate the anterior and posterior surface of the bladder. The cluster, c_2, which consists of about five fibers, innervates the posterior surface of the bladder and some of the proximal muscles of the second antenna. Judging from the course of c_2, the sensitive operculum is probably furnished with afferent fibers from c_2 rather than from c_1. The nerve-fibers to the bladder are probably mainly afferent.

Repeated observation could not duplicate, in *Cambarus,* Keim's affirmation (1915; and quoted by Stoll, 1925) that in the European crayfish there extends a nerve-fiber ("nervus glandulae viridis"), bilaterally, from the suboesophageal ganglion to the labyrinth. Keim considered Marchal's description of a renal innervation from the second antennal nerve-bundles as incorrect. Marchal, however, stated that he "could not find a nerve which passed directly to the antennal gland," i.e. without first going to the bladder. Neuwyler (1841) disagreed with the labyrinthic auditory hypothesis of his eminent predecessors, as regards the function of the "green glands," because he could never find a nerve-supply to the glands.[1] Wassiliew (1878), in one of the first papers on the histology of the decapod kidney, stated that no nerves could be seen to enter the kidney. The present writer's observations are in accord with Wassiliew in this respect. The absence of a nerve-supply at the kidney proper indicates that secretion by this organ is not influenced by the

[1] To Ernst Haeckel (1857) credit is due for first suggesting that the green glands are renal organs. Haeckel demonstrated the communication of the bladders with the exterior and with the glands by introducing metallic mercury into the bladders. He pointed out that the existence of an external orifice indicates that the liquid in the bladder is a secretion which is eliminated. This observation, together with Neuwyler's discovery of the tubule and Gorup-Besanez and Will's remark that guanine occurs in the green glands, led Haeckel to term these glands urinary organs. Gorup-Besanez and Will, however, did not state the concentrations of guanine in urine and blood.

nervous system. This is supported by Maluf, Clarke, and Thompson (1939), who were the first to show that, per unit volume of glomerular filtrate, the rate of secretion of various substances is identical in the denervated and normal mammalian kidney.

The Absence of a Valve between the Nephric Tubule and the Bladder

The epithelial cells of the bladder, except those of the most proximal portion, are never columnar. They may be highly vacuolated (Fig. 9, *A* and *B*) or plain (Fig. 9, *C*) in the same bladder. The physiological evidence indicates that the epithelium of the main body of the bladder is non-secretory (Maluf, 1941*b*).

Even though the columnar secretory epithelium of the distal half of the nephric tubule (Fig. 7, *dt* and Maluf, 1939) merges imperceptibly into the epithelium of the main body of the bladder (Maluf, 1939), the tubule as an organ ends abruptly (Fig. 7), since the epithelium of the bladder is not anastomosed and acutely involuted as is that of the tubule (Fig. 8). The distal orifice of the tubule can be readily observed *in situ* (Fig. 8) through the dorsal surface of the translucent distended bladder. There is no valve between the tubule and bladder (Fig. 8) and no evident constriction of the proximal end of the bladder. Sections show no valve or sphincter at the distal orifice of the tubule.

The bladder of a 14-gram animal normally can distend to a diameter of at least 8 mm. The hydrostatic pressure within the bladder must then be somewhat greater than that of a column of water 8 mm. high because the bladder is elastic (see above). This pressure is doubtless too low to interfere with the outward secretion of water for which evidence is presented in an accompanying paper (1941*a*).

The Absence of a Valve and Sphincter between the Coelomosac and Labyrinth

The entire series of sagittal and horizontal serial sections of two kidneys, fixed in formol-sublimate and stained with haematoxylin and methylene blue, was studied. No valve or fibers could be found. A sagittal section at the communication of the lumina of labyrinth and coelomosac is shown in Fig. 10.

The Epithelium of the Coelomosac

The epithelium of the coelomosac, like the rest of the nephron, is single-layered. The appearance of more than one layer throughout a

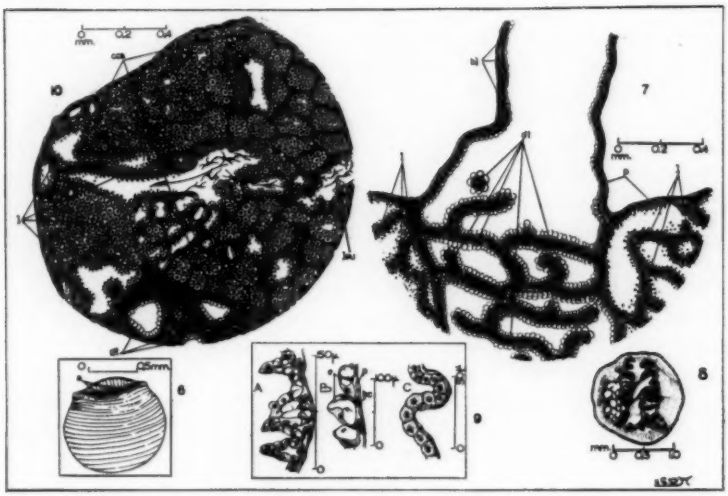

PLATE II

FIG. 6. Dorsal aspect of left nephropore, *a*, and membranous operculum. Note diagonal position of the nephropore. Animal, 22 grams.

FIG. 7. Sagittal section through the kidney showing communication of the distal extremity of the tubule, *dt*, with the bladder, *bl*. *l*, labyrinth; *p*, peritoneum. Solid black areas indicate blood-sinuses and blood-vessels. Animal, 35 grams.

FIG. 8. Dorsal aspect of the distal orifice of the tubule at its communication with the bladder.

FIG. 9. Sections of the main body of a single bladder. *bc*, blood-cells; *e*, epithelium of bladder; *p*, peritoneum. Animal, 35 grams.

FIG. 10. Sagittal section through the kidney showing communication of the coelomosac, *coe*, with the labyrinth, *l*. *dt*, distal portion of the tubule; *leu*, leucocyte. Solid black areas indicate blood-sinuses and blood-vessels. Animal, 35 grams.

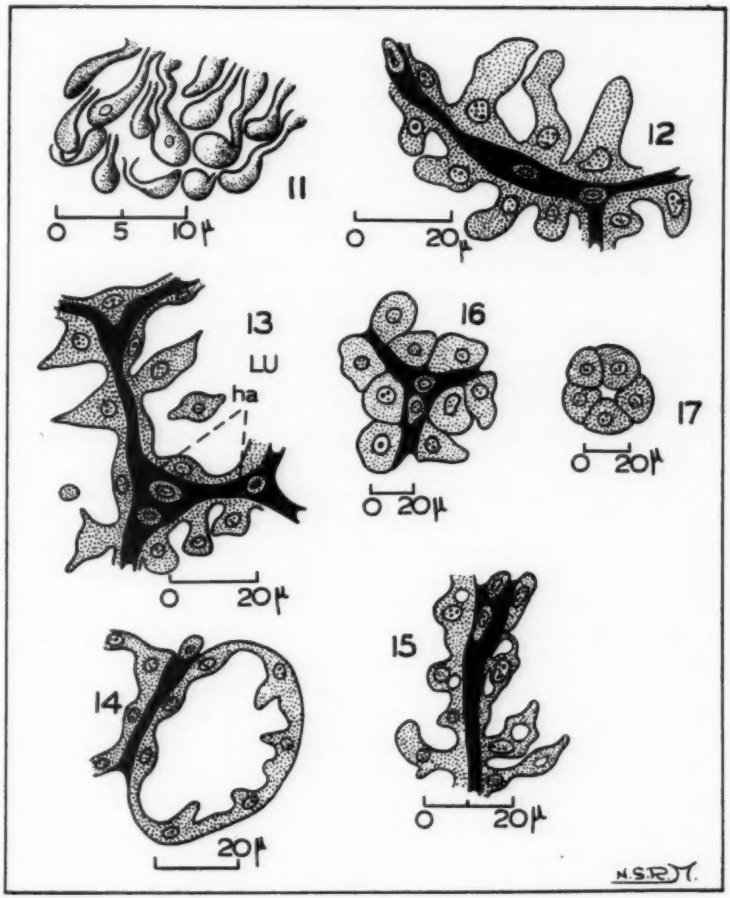

PLATE III

FIG. 11. "Living" portion of the coelomosac teased from the kidney in crayfish-saline and suspended in a hanging drop of crayfish-saline. The apical bulbous protuberances of the cells are shown in relief.

FIGS. 12 to 17. Epithelium of the coelomosac from medium-sized individuals. *ha*, haemocoele; *LU*, lumen of coelomosac. See text.

large part of the coelomosac, labyrinth, and tubule (Fig. 10) is doubtless due to tangential sectioning.

The nephron of the crayfish has no tenuous syncytium, such as the glomerular capsule of the vertebrate nephron. The epithelium of the coelomosac, the most promixal organelle of the nephron, approaches nearer to being a narrow syncytium than any other part of the nephron (Figs. 12–15). In several of the individuals examined, however, the cells were large and rounded throughout the coelomosac (Figs. 16 and 17; and Maluf, 1939).

The epithelium of the coelomosac varies considerably not only from one individual to another but often, too, in a single animal (Figs. 11–17). The cells may be either compact, rounded, and sometimes vacuolated, as in Figs. 16 and 17, or more or less squamous with large protuberances directed into the lumen (Figs. 12–15). The cells at the periphery may be rounded while those toward the center are protuberant; the reverse has never been found. Frequently, either the protuberant or the rounded cells occur exclusively. Both coelomosacs of an individual are always identical.

The histological methods have been described in the previous paper (Maluf, 1939). The protuberant type of cell is evidently not an artefact because it has been observed in teased-out "living" fragments in a hanging drop of crayfish-saline (Fig. 11). The composition of the saline is stated elsewhere (1941*a*).

Grabowska (1930) claimed that the secretion of the coelomosac consists of a discharge of cells in their entirety, i.e. "holocrine" secretion. If the cells are discharged as a whole, one would expect them to be substituted by mitosis. In not one instance, out of numerous coelomosacs examined, has the writer been able to find a mitotic figure. The evidence for a discharge of globules from the apical region of these cells is dubious because where rounded bodies have been seen "free," in the lumen of the coelomosac in sectioned preparations, these may have been merely sections of the bulbous tipped protuberances. In contrast to the rest of the nephron, the main lumen of the coelomosac generally contains numerous leucocytes (Fig. 10, *leu*).

Upon teasing the kidneys of a crayfish on one occasion, the coelomosacs were found packed with hard yellowish-brown irregular concretions the size of which showed that they could not have been intracellular. The largest stone was about 0.2 mm. in length. The material was insoluble in cold and hot water and in absolute ethyl alcohol. The alcohol decomposed the surrounding yellowish-brown organic material and the white stones readily fell apart, upon contact, into minute needle-like crystals which did not dissolve. The stones readily dissolved in

dilute HCl with energetic release of a colorless gas and were slowly soluble in 10 per cent NH_4Cl. There is very little doubt, therefore, that these concretions were $CaCO_3$. The individual had a highly melanized abdominal venter and, hence, must have possessed well-developed calcareous gastroliths. Twenty-one crayfish with gastroliths were examined and only one exhibited a similar condition. This was a single fairly large concretion in the coelomosac of only one kidney; other parts of the kidney did not contain any stones. About thirty animals with a light abdominal venter and without gastroliths were examined and in no instance was any concretion found in the kidneys.

The concentration of calcium in the blood of the crayfish and crabs remains fairly constant even immediately after molting (Paul and Sharpe, 1916; Damboviceanu, 1930), i.e. even when there is occurring, by way of the blood, a rapid transfer of calcium from the hepatopancreas and/or gut to the integument. Oesterlen (1840) has suggested that the formation of gastroliths may be a way of preventing a rise in the concentration of calcium in the blood. The above instances of renal calculi may be exceptions that prove the possible rule that one of the functions of the coelomosac is the secretion of calcium from the blood.

Weismann (1874) suggested that a blood-ultrafiltrate is formed through the coelomosac of the crustacean nephron just as Ludwig (1844) had presumed to occur through the glomerular capsule of the vertebrate nephron. Grobben pointed out that the relatively simple coelomosac of various amphipod crustaceans is attached to the integument by strands; this fact supports Weismann's belief inasmuch as effective resistance to blood-pressure would thereby be offered by the coelomosac, which would otherwise float in the haemocoele. Grobben also suggested that the location of the coelomosac between the antennal muscles in phyllopod Crustacea favors filtration. He nevertheless pointed out that, in copepod Crustacea, the coelomosac lies freely at the entrance to the homolateral second antenna and that these animals have no heart; he also drew attention to the fact that the phyllopod Crustacea have no heart and that it is therefore questionable whether, in such instances, filtration can occur and he ascribed the formation of urine in copepods and early-instar phyllopods to secretion by the tubule—a conception which had just begun to gain favor due to Heidenhain's (1874) experiments with the mammalian kidney.

Certain teleological evidence contra-indicates filtration through the coelomosac. Marshall and Smith (1930) and Marshall (1934) pointed out that when fishes migrated from freshwater, where they evidently arose, into the sea they had to conserve water. Some succeeded in losing their glomeruli while others are still doing so. The crayfish, how-

ever, has probably descended from a marine ancestor and is capable of compensating for water which diffuses inwardly through the gills (Maluf, 1937) by manufacturing a hypotonic urine through the agency of its nephric tubule. The crayfish nephron has a coelomosac but so does that of the lobster—a strictly marine relative. Because the osmotic pressure of the blood of the lobster is slightly hypertonic to that of the surrounding sea water (Cole, 1940), the lobster, unlike the crayfish, does not absorb water by diffusion from the exterior and hence does not have to maintain a steady state by an outward secretion of water. The lobster has either lost its nephric tubule or has never owned one. If the coelomosac were a filtration-organelle one would expect it to show some signs of regression in the lobster; but the coelomosac of this crustacean exhibits no evidence of being on the decline. Physiological evidence (Maluf, 1941*b*) indicates that the nephron of the crayfish is paramountly if not entirely a secretory organ.

Summary

1. The internal anatomical features surrounding the urinary outlet of the crayfish are described in detail for the first time.

2. Urine is retained in the bladders evidently by the ureteral syncytium, which is here described for the first time. There is no other way, conceivable to the writer, by which urine can be retained. Fibers do not insert on the operculum of the nephropore.

3. Urine is discharged by a localized rise in the haemocoelic pressure and can be expelled by direct action of the crop-gizzard on the bladders. Adequate electrical stimulation cannot cause contraction of the bladder but often evokes generalized motor activity.

4. Occasional abrupt spurts of urine, which were almost simultaneous from both nephropores, extended to the distance of a foot or more.

5. Destruction of the opercula before urinary collection has no rationale.

6. The bladder is innervated by fibers from the tritocerebral lobe of the brain. These fibers are doubtless mainly if not entirely afferent. The kidney is not innervated.

7. There is no valve between the nephric tubule and the bladder.

8. There is no valve or sphincter between the coelomosac and the labyrinth.

9. The epithelium of the coelomosac, the most proximal portion of the nephron, has been studied in detail. "Holocrine" secretion evidently does not occur because no mitotic figures could be found. The histological, chemical, and phylogenetical data contra-indicate filtration through the coelomosac.

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CHROMATIN BRIDGES AND IRREGULARITY OF MITOTIC COÖRDINATION IN THE PENTATOMID PEROMATUS NOTATUS AM. AND SERV.

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Introduction

A specimen of the pentatomid species *Peromatus notatus* obtained in 1937 presents such significant modifications of the orthodox course of meiosis, that a description and consideration of the most striking features seem warranted. As will be seen, the individual in question clearly is an exceptional case, but its departure from the normal is based on fundamental changes that have altered its mitotic mechanism in a very definite way. Apparently it is chiefly the relative timing of the various mitotic processes that has been affected, and the chromosomes and the spindle apparatus are, so to speak, out of step with each other. Their behavior under these conditions is of some interest in the analysis of mitosis in general.

Material

The specimen was caught on Barro Colorado Island in the Panama Canal Zone, in March, 1937. Its testes were fixed in B 15 within two hours of capture. It was close to the maximum size recorded for the species, in good condition, and very active.

Peromatus notatus, like the other six species of the genus, is strictly neotropical in its distribution. Examination of the sixteen specimens in the collections of the U. S. National Museum and the American Museum of Natural History shows that the species is subject to considerable variation in form and color. Variability in form is, however, more or less superficial and chiefly due to differences in the size and shape of the pronotal spines. The usual chestnut-brown color is replaced by green in some individuals from Panama (identified and labeled in the American Museum collection by H. G. Barber). It is a specimen of the latter type caught on Barro Colorado Island in 1941 that has served for comparison in the present study. It offers a typically pentatomid spermatogenesis which is almost indistinguishable from that of a specimen of *Peromatus truncatus* obtained in the same locality.

Spermatogonia

The spermatogonial divisions of the exceptional individual show no unusual features. The spindles conform to the common type, the chromosomes divide normally, and successive spermatogonial cell generations show no variation in chromosome number. The latter comprises the usual set of 14 chromosomes, in which one pair is a little larger and one pair somewhat smaller than the rest. The X is intermediate in size, whereas the Y is about as large as a member of the smallest pair (Fig. 1).

Meiotic Prophases to Diakinesis

Up to late diakinesis, the meiotic prophase stages conform to the usual pentatomid behavior. The sex chromosomes are heteropycnotic and frequently, though not always, appear joined from leptotene to diakinesis. There is a plasmosome which dwindles rapidly after the pachytene stage.

It is not until toward the end of diakinesis that the first unusual feature is encountered. Just as in other pentatomids, the two centers at this time move toward opposite sides of the nucleus. Both are in contact with the nuclear membrane and when they have reached their final position, the membrane underneath them is pulled or bulged outward.

This and the oval form of the nucleus, assumed in the direction of the centriolar axis, have frequently been noted (as early as 1891 by Henking). The point to be noted in this instance, however, is that the centers in the majority of cases are not on truly opposite points of the nucleus but are closer to each other on one side than on the other (Fig. 2). It is, of course, true that in other pentatomids also the position of the centers is not always geometrically exact, but the position here clearly is not accidental. This is borne out by the metaphase conditions that immediately follow the breakdown of the nuclear membrane.

The First Metaphase

The equator of the first spindle is in almost all cases displaced to one side, so that a line through the two centers does not represent the symmetrical axis of the mitotic figure as in other cases. In many cells all the chromosomes form a plate that lies to one side of the centriolar axis and hence the half spindle components are similarly displaced (Fig. 4). A few continuous fibres can sometimes be seen to stretch between the centers without such displacement, indicating their relative independence of the chromosomes. The latter rarely form a circle or round

plate, but constitute a semicircle with the two sex chromosomes usually but not always lying on the concave side (Fig. 3).

Despite this distortion of the mitotic apparatus, the tetrads divide in orderly fashion (Fig. 6) and the sex chromosomes undergo an equation division, just as in the normal *Peromatus* and other pentatomids. The peculiar configuration of the chromosome plate, however, is mirrored in the two daughter groups and may persist until middle anaphase (Fig. 5).

The initial movement of the dyads seems to occur without reference to the center and hence shows no effect of their askew position (Fig. 4). This is, of course, what might be expected since in nearly all cases known these first division stages of the chromosome appear to be autonomous.

The configuration of this first spermatocyte spindle challenges several interpretations concerning the mitotic mechanism. If the poles of the spindle are established by a mutual repulsion of two centrioles, it is very difficult to conceive of anything but a symmetrical spindle structure resulting therefrom. If the chromosomes assume their metaphase position because they react to forces from the poles, it is again not easy to understand why they should take such an "off center" position as they do. The conclusion is unavoidable that the mitotic conditions are affected by factors which normally are not present at this time.

First Anaphase to Second Anaphase

In the normal *Peromatus* as well as in most other pentatomids so far investigated, each of the centers carries two centrioles already at diakinesis. These two centrioles usually remain closely associated until telophase, though occasionally they have separated by some 15° before the end of anaphase (see, for instance, Paulmier's Fig. 29, 1899). The movement is quickened at telophase and before the second division is begun, the two centrioles are separated by 180°. There appears to be no exception to the rule that in Heteroptera the polar axis of this second division is at right angles to that of the first. This relation is especially striking in those cases where the interzonal connections of the first division continue to stain intensely, as in *Pachylis* (Fig. 8, and also those of other Heteroptera by Henking, 1891; Montgomery, 1898; and Paulmier, 1899).

The course followed in the present case is characterized by either one of two departures from the normal procedure just described. In about 75 per cent of the cells there is a marked precocity in the movements of the centrioles. Starting with little more separation than in normal cases, they diverge quickly after the early anaphase and in most cases have separated by 40°–45° before the anaphase movement of the chro-

mosomes has been completed (Fig. 7). Among the remaining cells about half show no such precocious separation of the centrioles, but the center as a whole may shift as much as 90° from the axial position of the first division (Fig. 9). In short, in such cases both centrioles assume the position of one of the poles of the second division, though the chromosomes are still in late anaphase of the first.

It was a matter of some surprise to find that in every such instance both centers moved to the same side of the anaphase cell. But this may simply be the consequence of the asymmetry of the first spindle which puts both centers closer to one side than the other to begin with. The two extremes of centriolar behavior are bridged by intermediate conditions which are not always easy to interpret. Thus the centrioles may succeed in separating after the center as a whole has begun to shift, or else one of the centrioles is for some reason held at the first pole and only the other moves toward its position for the second division (Fig. 7).

Whatever the type of variation may be, one point is held in common by all these cells. The processes that establish the achromatic figure of the second division are decidedly in advance of the corresponding steps in normal cells.

The precocity of the centers has marked effects on the behavior of the chromosomes. This is, perhaps, no more than might be expected, since they are still in the anaphase of the first division when the centers are already in process of establishing the mechanism for the second. The chromosomes show a definite response to the two poles which is manifested most strikingly in a tendency to divide again at this early

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PLATE I

Drawings made with Zeiss, 90 × objective and 20 × ocular. They were reduced ⅙ in reproduction.

FIG. 1. Three plates showing the 14 spermatogonial chromosomes.

FIG. 2. Diakinesis. The two centers are closer to each other on one side than the other.

FIG. 3. First metaphase. Autosomal tetrads arranged in semicircle, with *X* and *Y* on the inside.

FIG. 4. Side view of early anaphase, showing asymmetrical spindle. The autonomy of the initial separation of chromosomes is attested by lack of orientation toward the centers.

FIG. 5. Polar view of two sister groups in first anaphase, still showing typical arrangement.

FIG. 6. Middle anaphase of first division. The centrioles at each pole are separated less than usual.

FIG. 7. Upper pole of a late anaphase of first division. The two centrioles already have separated by about 45°, and there is no collocation of the chromosomes.

FIG. 8. Interphase in the coreid *Pachylis*, to show the characteristic relation of the second to the first spindle in the Heteroptera.

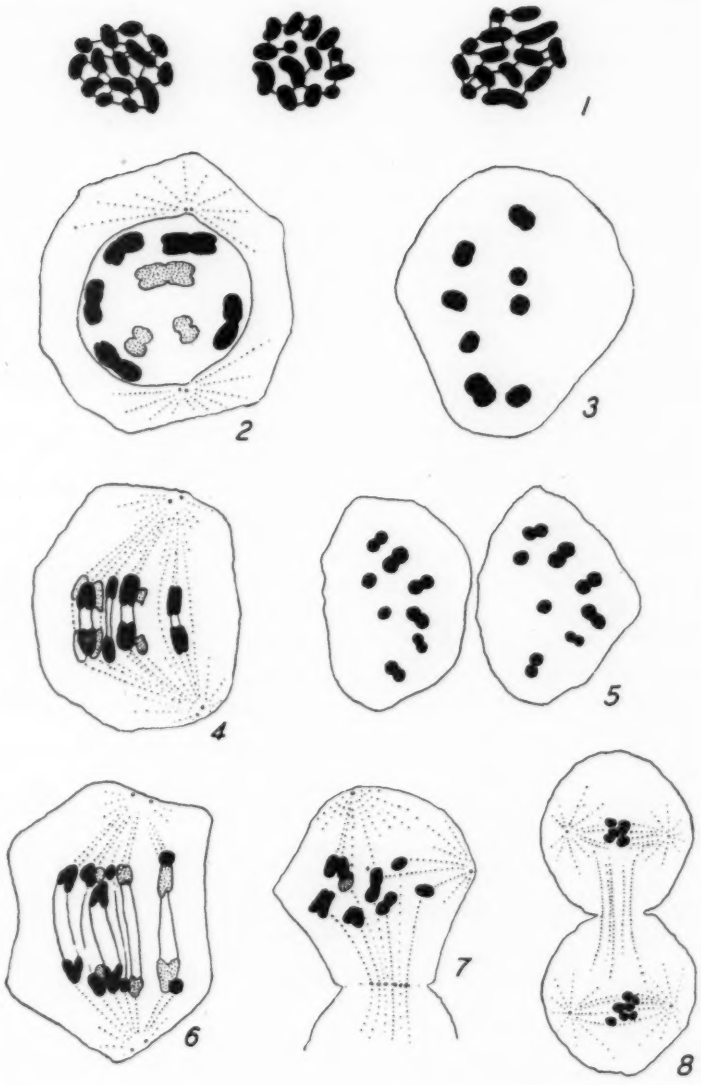

PLATE I

stage (Fig. 10). If this occurs before they have become dissociated from the interzonal connectives, such peculiar configurations as shown in Fig. 12 may result. In these as well as in less extreme cases the significant feature lies in the marked elongation of the chromosomes.[1]

This occurs despite the fact that the two chromatids of each dyad move in opposite directions toward the centrioles which are establishing a new axis. In other words, though the demarcation between the two chromatids is clearly indicated—as indeed it already is in diakinesis—and though the attenuation of the chromatids evidently betokens forces that tend to move them apart, they do not succeed in separating from each other (Figs. 10–13). The attenuating process continues until the chromosome body is torn into two pieces. The break apparently occurs at random and usually not in the natural line of separation between the chromatids (Figs. 13 and 14). Hence the amount of chromosome material distributed to each pole is variable and certainly not normal.

During this time the centriolar movement is completed. As a result the flexion that characterizes the spindles during the early part of this division disappears and the spindles of the late second anaphase are perfectly straight (Fig. 13).

PLATE II

FIG. 9. Late anaphase of first division. Each of the centers (both show two centrioles) has moved through 90° toward one pole of the second division.

FIG. 10. Centrioles of second division acting on chromosomes which are still in the condition of the first anaphase. (In Figs. 10, 11, and 12 only one of two sister cells is shown.) The demarcation between the chromatids is evident in several dyads.

FIG. 11. Second division showing attenuation, with chromatid demarcation showing in several dyads.

FIG. 12. Second division. The centrioles have separated relatively little, and the whole figure is strongly flexed as a result. Trace of interzonal connections of first division still showing at lower left.

FIG. 13. Late anaphase of second division. The spindle has straightened out. Chromatid demarcation still present in two of the dyads.

FIG. 14. Telophase of second division. There is no trace of collocation. The abnormality of the chromosome division is evident.

FIG. 15. Late telophase. Chromosomes still scattered and already becoming diffuse.

FIG. 16. Spermatid with four micronuclei, one Nebenkern, and one tail filament.

[1] It will be seen that the side of the chromosome presented toward the pole in the first division does not correspond to that of the second. This puzzling feature is, however, encountered in all Heteroptera and does not constitute a peculiarity of the present case. The explanation may lie in the fact that in the Hemiptera we are dealing with a "diffuse" instead of a localized kinetochore, as Hughes-Schrader and Ris (in press) have recently established.

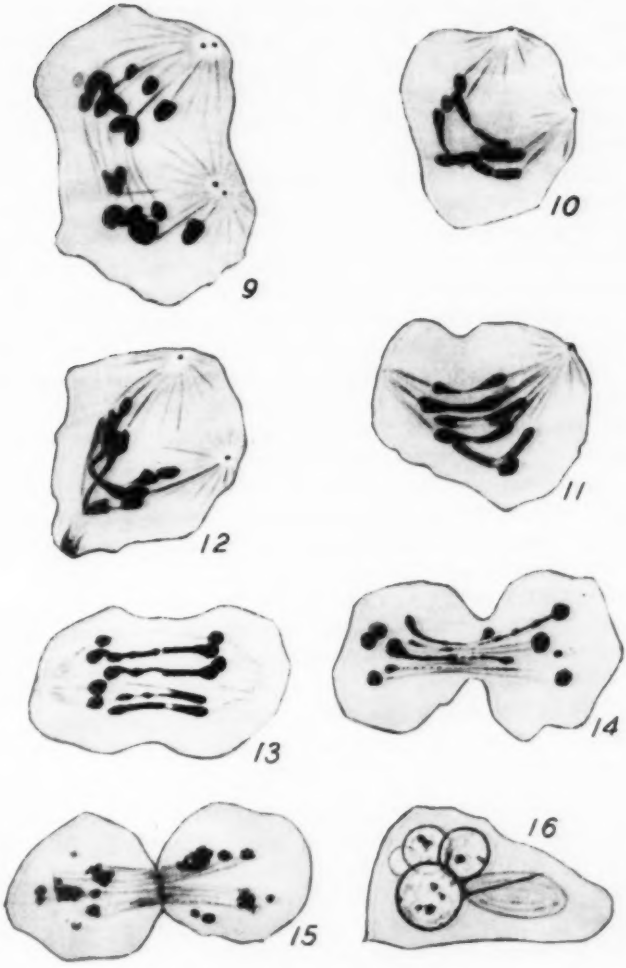

PLATE II

Second Telophase to Spermatozoa

Since the chromosomes of the first division are subject to the forces of the second division while they are still in anaphase, nothing can be said of their behavior under telophase conditions. Since there is only one centriole at each pole of the second division, no comparable centriolar disturbance takes place there and the chromosomes reach the telophase in every case.

Instead of the collocation of chromosomes that is typical of normal telophases, the chromosomes here actually tend to move further apart or to repel each other (Fig. 14). This tendency is not overcome even by the time that the chromatin becomes diffuse and as a result the products of the division lie more or less scattered in the cell (Fig. 15). Separate, small nuclei are formed from such masses of chromatin, and the spermatid is always a multinucleate cell (Fig. 16). In most cases only one Nebenkern is formed though in some instances two have been encountered. In no cell, however, does one find more than one axial filament and middle piece. These are associated with one of the nuclei which is not necessarily the largest one.

Apparently even the smallest of the nuclei takes steps toward the elongation that characterizes the formation of the sperm head. Later, however, there is much degeneration, though some of the sperms appear more or less normal.

The relative independence of mitotic phenomena in the cytoplasm and in the nucleus is attested by the fact that all the manoeuvers of the centers and the chromosomes do not hinder the division of the cytoplasm. Separate and complete cells, more or less equal in size, are found both after the first as well as the second division.

Discussion

The relationship of the significant features of this case is not always entirely clear, though it seems safe to assume that they are interconnected. They may be listed as follows: 1. The asymmetry of the first division figure. 2. The precocity in the behavior of the centers. 3. The attenuation and irregular division of the chromosomes in the second division. 4. The formation of multinuclear spermatids.

(1). The asymmetry of the first division figure is difficult to explain. If bipolarity is brought about merely by a mutual repulsion of two centers, the latter should be separated by 180° on the diakinetic nucleus and in the first metaphase. Again, the location of the chromosome plate, if it rests on a system of repulsive or attractive forces correlated with

those of the centers, should be on the axis formed by the latter. To explain the askew position of the chromosomes, it might be suggested that a primary spindle, comprised of fibres extending from pole to pole, arises before the chromosomes have formed a metaphase plate. This spindle then constitutes a core into which the chromosomes do not penetrate and hence they are disposed in the form of a semicircle around it. But such a hypothesis does not touch the root of the matter, which lies in the asymmetrical position of the centers themselves. And for this nothing more can be said than that a factor or force, probably extraneous to centers and chromosomes, is responsible.

(2). The extreme degree of separation of sister centrioles during the first division is clearly an indication of precocity in their cycle. Not so pertinent to this conception are those instances where the entire center, including both centrioles, moves to one of the poles of the second division (Fig. 9).

This might be attributed to the elongation of the spindle which pushes both centers around the periphery to one side. Precocity would there be expressed only in the development of astral rays and half-spindle fibres which actually appear to be growing at a time when in normal cases they are waning.

If, however, the movement of the undivided center is not thus accidental, its shift to the axis of the second division must mean that this pole is predetermined. This would imply that the centers are only secondarily concerned. The evidence hardly permits of extensive hypothetical considerations, but the early establishment of such a pole might involve forces that also are responsible for the asymmetry of the first division.

(3). But whether or not the centers are the primary agents in the determination of polarity, their direct influence on the chromosomes is not to be denied. This is strikingly shown in the premature second division, where it appears that the precocity of the centriolar processes is correlated with an exertion of forces that are normally not in evidence until a later stage. Their influence is indicated by the fact that the mitotic movement of chromosomes is toward the two centers from the very start. The autonomous separation of chromatids which takes place without reference to centers and which always comprises the first step under normal conditions, does not take place at all.

The attenuation of the chromosomes suggests that they are subjected to tensile forces. The failure of the chromatids to dissociate from each other under such conditions must then indicate that they are not yet completely ready when the centriolar forces are exerted thus precociously.

The lag does not lie in the chromosome proper, for in this as well as in normal cases all the chromatids are sharply demarcated from each other already in the preceding diakinesis (Fig. 2). That this demarcation persists into the second division is clearly shown in Figs. 10, 11 and 13, and the conclusion hence is unavoidable that a separation is prevented by other factors. The latter can be sought only in either the sheath or the matrix of the chromosomes, and it is therefore this constituent which is not yet ready for the division and holds the chromatids together.

It may be pointed out that the attenuation of chromosomes during division is not at all rare and that its cause is by no means always the same. It has been reported in cells that were subjected to X-ray or radium treatment. It is then usually correlated with a tendency of chromosomes to clump, and secondarily to translocations and inversions. Such cases are difficult to analyze since so many of the mitotic processes seem to be affected.

It has been described in tapetum cells (Steil, 1935) which show signs of degeneration. The attendant irregularities may well arise from an upset in the timing of the various mitotic processes as in the present case, but the necessary details of behavior that would justify such a conclusion are not available.

Bauer (1931) has reported it in *Tipula* and ascribes it to the presence of supernumeraries. The disturbance is there correlated with an adhesion of the chromosomes to each other.

It results from changes in the physical condition of the chromosomes, which in at least one case arise from the mutation of a single gene (Beadle, 1932). The "stickiness" which there characterizes the chromosomes seems to be caused chiefly by changes in the matrix and it is not impossible that the frequent attenuation during division is closely akin to that observed in *Peromatus*.

Lastly, it is a well-recognized characteristic of chromosome inversions which have resulted in dikinetic or dicentric chromosomes. Such "chromosome bridges" have played so striking a rôle in recent cytogenetic investigations that there has been a tendency to forget that not all chromatin bridges need be of the same nature. Thus Gentscheff and Gustafsson (1940) in their excellent study of meiosis in *Hieracium* utilize Beadle's conception that fragmentation of his maize chromosomes results from changes in the matrix, but quite ignore his explanation that his chromosome bridges were due to stickiness and increase in viscosity. Instead, they ascribe the very similar bridges in *Hieracium* to inversions and thus seem to agree with Darlington (1937, p. 320), who does not accept Beadle's convincing interpretation and states that "at anaphase

several bridges are found, showing that the changes include inversions."

It need hardly be pointed out that in the present case of *Peromatus notatus,* an explanation that rests on inversions is not tenable at all. This is already strongly indicated by the fact that the first division shows no bridges whatever, whereas they characterize all second divisions. To explain this on the basis of inversions would necessitate that two crossovers of a very specific type take place, and that these occur in the meiotic prophase of all cells. This would, moreover, result in a chromosome fragment which most assuredly is not present. Further, such a hypothesis would assume an orthodox, localized kinetochore, whereas here we are dealing with one of the diffuse type (Hughes-Schrader and Ris, in press). Finally, it must be remembered that an inversion bridge arises because bipolar tension is exerted upon a portion of a chromosome which does not include the natural line of demarcation between chromatids and which therefore can be divided only by tearing. In contrast, the bridges in *Peromatus* include the region where two chromatids, sharply demarcated from each other, are placed end to end. Dissociation therefore should and would follow quite normally without attenuation, if it were not hindered by the matrix or the sheath.

(4). Multinucleate spermatids arise because of the upset in the timing of mitotic processes. The chromosomes of the second division arrive at telophase when, in a sense, they are still in the anaphase condition. The mutual repulsion that characterizes them at the normal metaphase and anaphase, is therefore still encountered here when they have arrived at the poles. Hence there is the reverse of the usual collocation, the chromosome bodies are scattered singly or in small groups through the cell, and several micronuclei are found. The case for an irregularity in the timing of the centers is further supported by the fact that the actual division of the centrioles, albeit their movements are precocious, is in itself quite normal and only one middle piece and one axial filament are encountered in every multinucleate spermatid.

Conclusion

The nature of the case makes it rather futile to speculate on the origin of the meiotic abnormalities just described. Practically nothing is known about the ecology of the genus, and the possibility of interracial and interspecific crosses is purely hypothetical.

Clearly, however, the case is an exceptional one for the species. The conditions basically affect the production of normal sperms and can have no survival value. Indeed, the rather orthodox course of spermatogenesis in other specimens of *Peromatus* renders this certain.

But so far as this individual is concerned, the abnormality is a deep-seated one since the absence of normal spermatids indicates that it has persisted for some time. The conditions strongly suggest that at least one of the mitotic processes has fallen out of step and that coördination with the other processes becomes progressively more difficult in the successive cell generations, from spermatogonia to spermatids. The disturbance has no visible effect on the spermatogonia; has a well-defined influence on the spindle mechanism of the first division without, however, upsetting the essential aspects of orderly chromosome division; renders impossible a normal distribution of chromosomes in the second division; and culminates in spermatids that are definitely abnormal.

Summary

1. The abnormal course of meiosis in a specimen of *Peromatus notatus* is characterized by a series of well-defined irregularities.

2. The spindle of the first division shows both centers to one side of the geometrical axis and the metaphase plate displaced to the opposite side.

3. Before the chromosomes of the first division have reached the poles, they are subjected to the forces involved in the second division.

4. The effect on the chromosomes is to attenuate them without bringing about a normal division. The resulting configurations simulate inversion bridges, though that is quite clearly not their nature.

5. The spermatids receive varying amounts of chromosome material and are multinucleate.

6. It is suggested that this abnormal meiosis is due to an irregularity in the timing of one of the mitotic processes. The indications are that this process involves the movement of the centers.

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